Development of Methane Fermentor for Restraining Ammonia Inhibition

著者	WANG Qinghong
内容記述	Thesis (Doctor of Environmental Studies)--University of Tsukuba, (A), no. 6320, 2012.7.25 Includes bibliographical references (leaves 81-96)
year	2012
URL	http://hdl.handle.net/2241/117480

Development of Methane Fermentor for Restraining Ammonia Inhibition

May 2012

Qinghong WANG

Development of Methane Fermentor for Restraining Ammonia Inhibition

A Dissertation Submitted to

the Graduate School of Life and Environmental sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Environmental Studies

(Doctoral Program in Sustainable Environmental Studies)

Qinghong WANG

Abstract

Anaerobic digestion of swine wastes is an attractive practice in which both pollution control and energy recovery can be achieved. Nowadays, anaerobic digestion becomes a promising process due to its beneficial output including renewable energy production and mitigation of pollutant emissions. However, the high concentration of ammonium in manure and the produced ammonia as a common metabolic end product during the anaerobic digestion of protein-containing substrates often cause the inhibition of anaerobic digestion process, because it is toxic to anaerobes.

Several methods are available for eliminating ammonium inhibition in the anaerobic digestion process including adding adsorption materials, chemical precipitation, ammonia stripping, and biological ammonium removal. Given its advantages and limitations, adding adsorption materials has been recognized as a favorable technology with the advantages of convenient and economic. Furthermore, the ammonium saturated material can be recycled as fertilizer which fulfills the requirements of sustainable development. Therefore, the aim of this study is to develop an anaerobic bioreactor integrated with adding adsorption materials for eliminating ammonium inhibition and improving anaerobic digestion efficiency.

Firstly, a zeolite fixed bioreactor was developed for anaerobic digestion of ammonium-rich swine waste. This section was mainly focus on the configuration of the bioreactor by comparing the performance of anaerobic digestion in the zeolite sunken bioreactor and without zeolite bioreactor. The new bioreactor exhibited good

performance, with startup time on the 14th day and methane production of 178.5 ml/g-VS during all 32 days of the experiment at 35 ºC. This bioreactor significantly shortened startup time, enhanced methane gas yield more than two fold and made COD removal more efficient than under the other models.

After that, a porphyritic andesite (WRS) was tried to use as an ammonia adsorbent and bed material for the anaerobic digestion process. To improve its ammonia adsorption capacity, a calcium salt treated and calcination method was developed. Scanning electron microscope (SEM) and Brunauer–Emmett–Teller (BET) surface area analyses were performed to characterize the Ca-modified WRS, and adsorption isotherms and kinetics were investigated to clarify the adsorption mechanism. The ammonium adsorption process was explained well with a pseudo-second-order kinetic model. The specific surface area of the Ca-modified WRS was determined to be 4.56 sq. m/g, and the maximum NH_4^+-N adsorption capacity was determined to be 45.45 mg/g. These values are improvements over those of natural WRS. The ammonium adsorption capacity remained constant at a pH range from 5.0 to 9.0, which indicates that Ca-modified WRS is a promising material for various applications.

Then the modified WRS fixed bioreactor was set up and the anaerobic digestion of ammonium-rich swine wastes in bioreactors with modified WRS, natural WRS, calcium chloride and no additives was investigated. The modified porphyritic andesite bioreactor exhibited the best performance, with start-up time on the 7th day, methane yield of 359.71 ml/g-VS, and COD removal of 67.99% during all 44 days of the experiment at 35 ℃. The effective ammonium adsorption and essential ions dissociation for microorganisms by modified WRS, as well as the immobilization of microbial on the surface of the modified WRS play a great role on the high efficiency anaerobic digestion of ammonium-rich swine waste.

Contents

Figures

Tables

Chapter 1 Introduction

1.1 Background

Nowadays, with the increasing demand of pork, intensive swine breeding constitutes one of the most important agricultural activities in the world. In Canada, there were 14.7 million hogs in 2002, according to the Statistics Canada-Census of Agriculture. In China, there were 463 million hogs in 2008, which was 5.17% higher than previous year [1]. In the southeastern USA, frequently over 20,000 hogs were raised indoors at single locations [2]. In Cuba, there were piggeries with around 3000 animals in each [3].

It is reported that average manure production per hog for dry feeder barns was 4.03 l [4]. The high strength and large volume of piggery wastes with a high concentration of organic matter, nutrients, trace elements and a variety of pathogenic are detrimental to the environment and also represent a potential hazard to human and animal health. Eutrophication of lakes, reservoirs and estuaries has raised realistic questions regarding the presence of nitrogen, phosphorus and carbon in runoff from swine production areas [5-7]. It is reported that 69% of contaminated rivers and streams was from animal wastes which was identified as the largest contributor to water pollution [8]. Therefore, the disposal of the large quantities of wastes by designing and building purification systems is very important and necessary.

1.2 Anaerobic digestion process

Anaerobic digestion process with the advantages of low sludge accumulation, low nutrient requirements and high efficiency of methane production has been widely used as an attractive waste treatment practice [9]. In contrast to aerobic waste processes, most of the nutrients remain in the treated material and the end-product can be used as agricultural fertilizer [10]. Moreover, produced methane can be used as clean energy to replace fossil fuel or petroleum and CO_2 emissions can be reduced thereby.

1.2.1 Theory of anaerobic digestion

Anaerobic digestion is a biological process in which organic matter is degraded to methane under anaerobic conditions. A diversity of micro-organisms are involved in many steps of anaerobic degradation of complex substrates, such as swine waste, the efficiency of the process depending on the operating conditions [11]. The end products of the process are mainly methane and carbon dioxide. The overall conversion process of complex organic matter into methane and carbon dioxide can be divided into four steps: hydrolysis, acidification, acetogenesis, and methanogenesis [12], as shown in Fig. 1.1.

The pathway of anaerobic digestion process can be described as the hydrolytic bacteria hydrolyzes and converts the organic compounds into volatile fatty acids with the simultaneous production of hydrogen and carbon dioxide, the acidogenic and acetogenic bacteria produce various organic acids and convert the organic acids to acetic acid, respectively, and the methanogenic bacteria produce methane and carbon dioxide, either from acetate or from H_2 and CO_2 [13].

In an anaerobic digester, the four processes occur simultaneously. Hydrolysis is a relatively slow process and generally it limits the rate of the overall anaerobic digestion process. The second step is acidogenesis or acidification which is able to metabolise organic material down to a very low pH (around 4). In the acetogenesis-step, the products of the acidification are converted into acetic acids, hydrogen, and carbon dioxide by acetogenic bacteria. The first three steps of anaerobic digestion are often grouped together as acid fermentation. It is important to note that in the acid fermentation, no organic material is removed from the liquid phase: it is only transformed into a form suitable as substrate for the subsequent process of methanogenesis. In the final step of the anaerobic digestion process, organic material is removed. The mainly products of acid fermentation (acetic acid) are converted into CO_2 and CH_4, which are desorbed from the liquid phase.

1.2.2 Theoretical calculation

The theoretical CH_4 production B_u was calculated from Bushwell's [14] formula

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 \quad (1)$$

$$Bu = \frac{\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}\right)22.4}{12n+a+16b} \text{ l } CH_4/g^{-}VS \quad (2)$$

The main chemical composition of swine waste was lipid, protein and carbohydrate, which can be represented by lipid of $C_{57}H_{104}O_6$, protein of $C_5H_7O_2N$, and carbohydrate of $C_6H_{10}O_5$. The corresponding anaerobic digestion can be summarized as:

$$C_{57}H_{104}O_6 + 28H_2O \rightarrow 17CO_2 + 40CH_4 \quad (3)$$

$$C_5H_7O_2N + 4H_2O \rightarrow HCO_3^- + 1.5\ CO_2 + 2.5\ CH_4 + NH_4^+ \quad (4)$$

$$C_6H_{10}O_5 + H_2O \rightarrow 3CO_2 + 3CH_4 \quad (5)$$

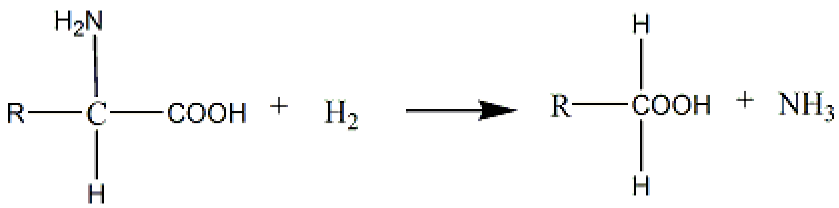
The theoretical CH_4 production from lipid, protein, and carbohydrates is 1014, 496, and 415 l CH_4/ kg-VS, respectively.

1.3 Ammonium inhibition problems

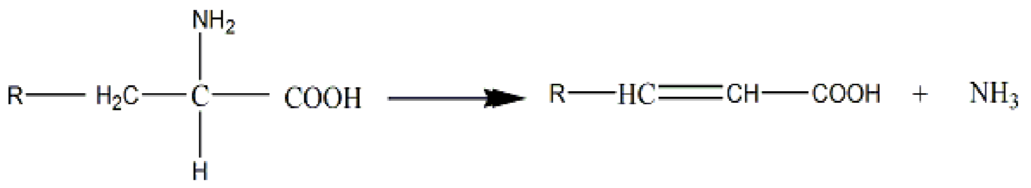
1.3.1 Biochemical pathways of ammonia production

A quantity of ammonium will be generated from an anaerobic biodegradation of organic substrate, such as protein which is shown in equation (4). A protein is a long, complex chain of alpha-amino acids, linked by peptide bonds. During hydrolysis, shorter chains of amino acids are produced as intermediate compounds and then are further hydrolysed to ammonium. Besides proteins, other nitrogenous compounds including phospholipids, nitrogenous lipids and nucleic acids are sequentially digested by the anaerobic microorganisms, and eventually ammonium is produced.

The anaerobic deamination of alpha-amino acids may proceed with reduction, to form fatty acids or without reduction to form saturated fatty acids, as presented below [15]:



(6)

 (7)

where R is a group of molecules, characteristic to each amino acid.

1.3.2 Mechanisms of ammonium inhibition

Anaerobic digestion of swine manure as sole substrate has been shown to be unsuccessful in many cases due to the ammonium inhibition. Several mechanisms for ammonium inhibition have been proposed, such as hydrophobic ammonia molecule diffuse passively into the cell causing proton imbalance and the change of intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction [16]. The ammonium inhibition study conducted by Wiegant and Zeeman [17] indicated that the high total ammonia concentration severely affects the growth rates of hydrogen utilizing methanogens and acetate appears quickly upon increase in total ammonia nitrogen concentration. It is hypothesized that an intermediate compound accumulates during inhibition of hydrogen and propionate.

Koster and lettinga [18] indicateds that under mesophilic conditions, the maximum methanogenic activity was found to be unaffected at total nitrogen concentration of 680 mg/l. However, the methanogenic activity decreased to about 75% at NH_4^+-N concentration of 759 mg/l. The methanogenic activity further declined as the NH_4^+-N concentration was increased.

Among the four types of anaerobic microorganisms (hydrolytic, acidogenic, acetogenic, and methanogenic bacteria), the methanogens are the least tolerant and the most likely to cease growth due to ammonium inhibition [19]. As ammonia concentrations were increased in the range of 4051–5734 mg N/l, acidogenic populations in the granular sludge were hardly affected while the methanogenic population lost 56.5% of its activity [20]. There are two main groups of methanogens involved in anaerobic digestion: acetoclastic and hydrogenotrophic, which account for 70% and 30% of methane output, respectively [21]. Acetoclastic methanogens produce methane while consuming acetate or acetic acid, and hydrogenotrophes utilize hydrogen and carbon dioxide to create methane. Some research based on the comparison of methane production and growth rate indicated that the inhibitory effect was in general stronger for the aceticlastic than for the hydrogenotrophic methanogens [22, 23]. Among the methanogenic strains commonly isolated from sludge digesters, i.e. *Methanospirillum* hungatei, *Methanosarcina* barkeri, *Methanobacterium* thermoautotrophicum, and *Methanobacterium* formicicum, *Methanospirillum* hungatei was the most sensitive, being inhibited at 4200 mg N/l [24].

1.4 Factors controlling ammonia inhibition

1.4.1 Ammonium concentration

It is generally believed that ammonia concentrations below 200 mg/l are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms [25]. A wide range of inhibiting ammonium concentrations has been reported in the literature. McCarty [26] reported that NH_4^+-N concentration in excess of 300 mg/l was expected to be toxic to the microorganism. Zeeman et al [27] reported an initial inhibition of anaerobic digestion at NH_4^+-N concentration of 1700 mg N/1 in the thermophilic reactor (50 ℃). Hashimoto [28] found ammonium inhibition at about 2500 mg N/l for both mesophilic and thermophilic reactors when the reactors were not previously acclimatized. However, the corresponding value was 4000 mg N/1 for thermophilic reactors previously acclimatized to ammonia concentrations between 1400 and 3300 mg N/1. Hobson and Shaw [29] reported that 235 mmol/l (3290 mg N/l) ammonium completely prevented the growth of *Methanobacterium formicicum* in the pure culture. Angelidaki and Ahring [22] reported that NH_4^+-N concentrations of 4000 mg/1 or more inhibited thermophilic digestion of cattle manure. Much higher inhibitory levels have been reported by other authors. Van Velsen [30] showed in batch experiments, with inoculum adapted to high concentrations of ammonia, that methanogenesis occurred after a lag phase at ammonia concentrations as high as 5000 mg N/1. Jarrell et al [24] reported that some methanogenic strains are inhibited at ammonium concentration of 4200 mg N/l, while others are resistant to higher than 10000 mg N/l. The significant difference in inhibiting concentration can be attributed to the differences in substrates and inoculum, environmental conditions, and acclimation periods.

1.4.2 pH value

During treatment of waste containing high concentrations of NH_4^+-N, pH affects the growth of microorganisms as well as the composition of NH_4^+-N. Ammonia gas reacts with water to produce ammonium and hydroxide ion according to the following pH dependent relationship:

$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$ (8)

The unionized species is often called free ammonia, since it exists as molecule in solution. Since the free form of ammonia has been suggested to be the actual toxic agent, an increase in pH would result in increased toxicity [31] because of the shift to higher free ammonia to ionized NH_4^+ ratio at higher pH. The concentration of free ammonia nitrogen can be calculated by using the following formula [32]:

$$\text{Free ammonia} = \frac{Total\ ammonium}{1+10^{(pKa-pH)}} \quad (9)$$

$$pK_a = 0.09018 \ \frac{2729.92}{T+273.15} \quad (10)$$

where pK_a is the dissociation constant for ammonium ion 8.95 at 35 ℃, T is the temperature, ℃.

Fig.1.2 is the relationship between free ammonia and ammonium ions in the thermophilic conditions (55 ℃). As can be seen in this figure, for a total ammonium concentration of up to 1000 mg/l, the free ammonia will remain below the maximum tolerable level of 55 mg/l if the pH is 7.2. However, if the digester operates at a pH of 7.5, ammonia inhibition may occur at total concentration as low as 400 mg/l. Therefore, to limit the inhibitory effect of free ammonia on anaerobic microorganism, it is desirable to control pH at a low value.

Process instability due to ammonia often results in volatile fatty acids (VFAs) accumulation, which again leads to a decrease in pH and thereby declining concentration of free ammonia. The interaction between free ammonia, VFAs and pH may lead to an “inhibited steady state”, a condition where the process is running stably but with a lower methane yield [22]. Control of pH within the growth optimum of microorganisms may reduce ammonia toxicity [33]. Zeeman et al [27] reported that reducing pH from 7.5 to 7.0 during thermophilic anaerobic digestion of cow manure increased the methane production by four times. From Braun et al.'s report [34], during anaerobic digestion of liquid piggery manure (pH = 8), VFAs accumulated to 316 mg/l. Adjustment of pH to 7.4 led to reutilization of VFAs and lowered VFAs concentrations to 20 mg/l. The better performance at pH 7.4 has been attributed to the relief of ammonia-induced inhibition at low pH.

1.4.3 Temperature

Both microbial growth rates and free ammonia concentration are affected by temperature. An increased process temperature in general has a positive effect on anaerobic digestion because it will lead to give faster reaction rates, higher gas production, and higher rates of the destruction of pathogens and weed seeds. However, the thermophilic process is more sensitive to environmental changes than the mesophilic process [35]. Several authors have found that anaerobic digestion of organic wastes with a high concentration of ammonia was more easily inhibited and less stable at thermophilic temperatures than at mesophilic temperatures [34, 36]. Thermophilic digestion of cow manure at 50 ℃ with total NH_4^+-N above 3000 mg/l was found to be very difficult [37]. The effect of temperature on methane production kinetics in anaerobic digestion on cattle manure was studied by Sanchez et al. [38] They used two laboratory-scale batch complete mixed reactors which were operated at mesophilic and thermophilic temperatures. The results indicated that total methane production rates at mesophilic temperature were slightly lower than those at thermophlic temperature. An increase in operating temperature from 37 ℃ to 60 ℃ in anaerobic digesters with a high ammonia concentration provided intensified inhibition caused by free ammonia, as indicated by an decrease in biogas yield [39, 40].

Although thermophilic anaerobic digestion could potentially have faster biogas production rates, heating the system requires a large amount of energy and could not be economically viable. It is also difficult to maintain the system efficiency because biological community becomes more sensitive at higher temperature.

1.4.4 Acclimation

Acclimation is another factor that influence the degree of ammonium inhibition. The first reports dealing with adaptation of methanogens to ammonia by exposing them to slowly increasing concentrations was the sludge digestion study by Melbinger and Donnellon [41]. After that, the adaptation of methanogens to a wide variety of potentially inhibitory substances has been reported [21, 36]. The adaptation may be the result of internal changes in the predominant species of methanogens, or of a shift in the methanogenic population [27]. Once adapted, the microorganisms can retain viability at concentrations far exceeding the initial inhibitory concentrations [33, 42]. Koster and Lettinga [20] reported that while unacclimated methanogens failed to produce methane at 1900 - 2000 mg N/l, but produced methane at 11000 mg N/l after adaptation. Hashimoto [28] observed that ammonia inhibition began at about 2500 mg/l and 4000 mg/l for unacclimated and acclimated thermophilic methanogens, respectively. Successful operation of anaerobic filters has been achieved at 6000 mg/l and 7800 mg/l after adaptation [43]. Parkin and Miller [36] reported that NH_4^+-N levels as high as 8000 - 9000 mg/l could be tolerated with no significant decrease in methane production after acclimation. The experiments clearly demonstrated the possibility of obtaining stable digestion of manure with ammonia concentrations exceeding 5000 mg N/l after an initial adaptation period. However, the methane yield was lower than that for reactors with a lower ammonia load.

Although with adaptation of methanogens to ammonia methanogens could produce methane at a high concentration of NH_4^+-N, methane yield was low and long acclimation period is required.

1.5 Methods for ammonium removal

Ammonium removal is necessary for anaerobic digestion of ammonium-rich organic wastes. Because even methanogens is able to growth under high concentration of NH_4^+-N by pH or temperature control or acclimating to ammonia for a long period, the methane yield is low and anaerobic digestion efficiency is not desirable. Ammonia removal not only enhances anaerobic digestion performance by decreasing the ammonia concentration in the feed but also maintain the ammonia concentration of the effluent in a range that is safe for subsequent biological processes. Several different methods have been proposed for ammonia removal, these methods can be divided into physical, chemical and biological method.

1.5.1 Physical methods

Ammonia air stripping in combination with absorption has been considered a good option when treating different types of waste: liquid fraction of dewatered sewage sludge [44]; urea fertilizer plant wastes [45], landfill leachate[46] and condensates from a sugar beet factory [47]. In all these cases, the process was performed at high pH values. Liao et al. [48] studied ammonia air stripping from pig slurry at room temperature. At this temperature, a high pH (10.5 - 11.5) was required to obtain high ammonia removal efficiencies. In order to obtain a high pH, a large amount of alkali is needed in the ammonia stripping process. The resulting high concentration of cations might readily affect the activity of microbes and interfere with their metabolism in the subsequent biological processes. For example, sodium ion concentrations of 5, 10, and 14 g/l led to inhibition by 10%, 50% and 100%, respectively, with respect to the inhibition of methanogens in an anaerobic granular biomass at mesophilic temperature and neutral pH [49]. However, if air stripping is performed at high temperature, the high buffering capacity of the pig slurry could probably maintain pH at the needed value, and the amount of alkali could be reduced. The main limiting factor for ammonia air stripping at high temperature is the availability of a cheap thermal energy source. Besides that, with an increase of temperature causing a greater fraction of free ammonia, and also has a physical effect in increasing the saturated vapour pressure of the free ammonia, the toxicity to microorganisms will be caused thereby.

Adding ammonia-adsorption materials is also wildly used, these materials includes bentonite, zeolite, activated carbon, glauconite and so on. Angelidaki and Ahring [50] used bentonite and bentonite-bound oil (BBO) to improve the anaerobic thermophilic digestion of cattle waste. They found that the major effect of bentonite and BBO was not through a direct ammonia removal, but through an increasing resistance to ammonia inhibition. Hansen et al.[40] reported that the addition of activated carbon or glauconite or both of these in pig waste improved the thermophilic anaerobic digestion and methane production. They showed that the concentration of sulphide has to be taken into account when studying ammonia inhibition of manure. The enhanced anaerobic digestion performance was mainly explained by a reduction of the sulphide content by adsorption to the activated carbon or precipitation as ferrous sulphide. Of the possible materials, zeolite is especially useful in improving the methane production of ammonium-rich biomass because it can facilitate the selective adsorption of ammonia and cation exchange in ammonium [51]. In addition, the following characteristics also make zeolite promising for use in anaerobic digestion: (1) high capacity to immobilise microorganisms as a porous surface; (2) ability to withstand high temperature and acid; and (3) wide distribution, economic and obtainable [52]. The influence of zeolite concentration, zeolite dosage procedure, and zeolite type on the anaerobic digestion of ammonium-rich organic wastes has been researched by several authors. Tada et al. [53] have illustrated that natural mordenite has a synergistic effect on Ca^{2+} supply and NH_4^+ removal during the anaerobic digestion of ammonium-rich organic sludge. Montalvo et al. [54] have shown that adding natural zeolite on a daily basis is the most appropriate way to promote the continuous anaerobic digestion of swine wastes in terms of COD removal efficiency and methane production. Milán et al. [55] have suggested an optimum natural zeolite dosage of 2 - 4 g/l with an initial concentration of NH_4^+-N 410 mg/l.

According to Kotsopoulos et al. [56], natural zeolite at doses of 8 and 12 g/l swine waste was better than doses of 0 and 4 g/l in terms of methane production in the context of thermophilic anaerobic digestion where the initial concentration of NH_4^+-N was less than 300 mg/l.

1.5.2 Chemical methods

One simple and rapid method to remove and recover nitrogen is its crystallization in the form of struvite (magnesium ammonium phosphate, or $MgNH_4PO_4 \cdot 6H_2O$), which may be utilized as a valuable source of slow release fertilizer due to its solubility characteristics [57]. Struvite crystallizes as a white orthorhombic crystalline structure, which is composed of magnesium, ammonium and phosphate in equal molar concentrations [58].

Rico et al. [59] developed a physical–anaerobic–chemical process for treatment of dairy cattle manure. The removal of nitrogen by struvite precipitation, methane production and obtention of valuable fertilizers from dairy manure could be obtained. Ryu and Lee [7] investigated the pretreatment of NH_4^+-N by struvite precipitation in treating swine wastewater. In that study, the struvite precipitation enhanced the biological COD removal performance by reducing the toxic effect of free ammonia. Marti et al. [60] investigated the phosphorus precipitation in an anaerobic digestion pilot plant. The results showed significant phosphorus precipitation as struvite (58% of the fixed phosphorus) and a low precipitation of calciumphosphates (15%), forming mainly hydroxyapatite. A study reported in which struvite precipitation was carried out during the anaerobic digestion of a food waste [61]. The results indicated that struvite precipitation obtained by addition of Mg^{2+} during anaerobic digestion led to 67% and 73% N and P removals, respectively. Krylova et al. [62] reported that addition of phosphorite ore could prevent ammonia inhibition in the anaerobic digestion of poultry manure, supposedly by either immobilizing methanogens on the mineral grains, which increases the buffering capacity of the medium, or by exchanging ammonium ions for cations such as K, Ca, Mg. The high efficient of chemical precipitation for ammonium removal can be obtained, but chemicals are needed additionally and the actual operation is difficult.

1.5.3 Biological methods

Anaerobic ammonia oxidization (Anammox) is a recently discovered anaerobic process where ammonium is oxidized to nitrogen gas using nitrite as the electron acceptor [63]. The stoichiometry of the Anammox reaction is given in Eq. (11).

$$NH_4^+ + 1.32\ NO_2^- + 0.07\ HCO_3^- + 0.13\ H^+ \rightarrow$$

$$1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O + 0.07\ CH_2O_{0.5}\ N_{0.15} \qquad (11)$$

Dong and Tollner [64] evaluated anammox and denitrification during anaerobic digestion of poultry manure. The liquid with high ammonia/ammonium concentration in the anaerobic process was removed from the bottom. Half of the ammonium in the liquid was partially nitrified to nitrite firstly and the liquid would subsequently be recirculated to the anaerobic digester. About 13 - 22% ammonium removal was observed by converting to N_2 through Anammox using nitrite as electronic acceptor in the anaerobic digester. Waki el al. [65] applied Anammox to wastewater from an activated sludge reactor treating swine manure and from the anaerobic treatment followed by trickling filter of swine manure. They concluded with successful results and proposed partial nitrification as pretreatment for the practical application of Anammox. Karakashev et al. [66] employed Anammox treatment after partial oxidation in a multi-stage treatment treating swine manure, achieving nitrogen removals of up to 90%. Yang et al. [67] investigated simultaneous removal of ammonium and sulfate from an anammox process in an anaerobic digestion bioreactor filled with granular activated carbon. However, there are some limitations of the process. Anammox bacteria are very sensitive, which are inhibited by more than 0.94 mg dissolved O_2/l [68]. The process is inhibited by NO_2^- concentration around 0.1 g N/l working under continuous operation, but is 50% inhibited by NO_2^- concentration of 0.36 g N/l [69]. Although the biological method is beneficial to environment and sustainable development, however, if high activity of the bacteria is kept severe conditions need to be maintained such as pH, temperature, dissolved oxygen, and so on.

1.6 Objectives of the research

The introduction part has concluded that anaerobic digestion is a very promising alternative treatment technology for swine waste. When used in practice, however, its low efficient and long lag phase is often occurred. Much of this difficulty could be contributed to the presence of excessive inhibitory compounds of ammonium. The purpose of this study is to resolve the problem of ammonium inhibition for anaerobic digestion of ammonium-rich swine waste. The specific objectives are listed as follows:

(1) Based on the easy operation and economy, to develop a fixed-zeolite bioreactor for use in the anaerobic digestion of ammonium-rich swine wastes.

(2) To further improve the activity of microorganism, a modified porphyritic andesite with high ammonium removal capacity and cation dissociation ability is to be developed.

(3) The performance of anaerobic digestion of ammonium-rich swine wastes is to be investigated by using the modified porphyritic andesite as an ammonium adsorbent and a bed material.

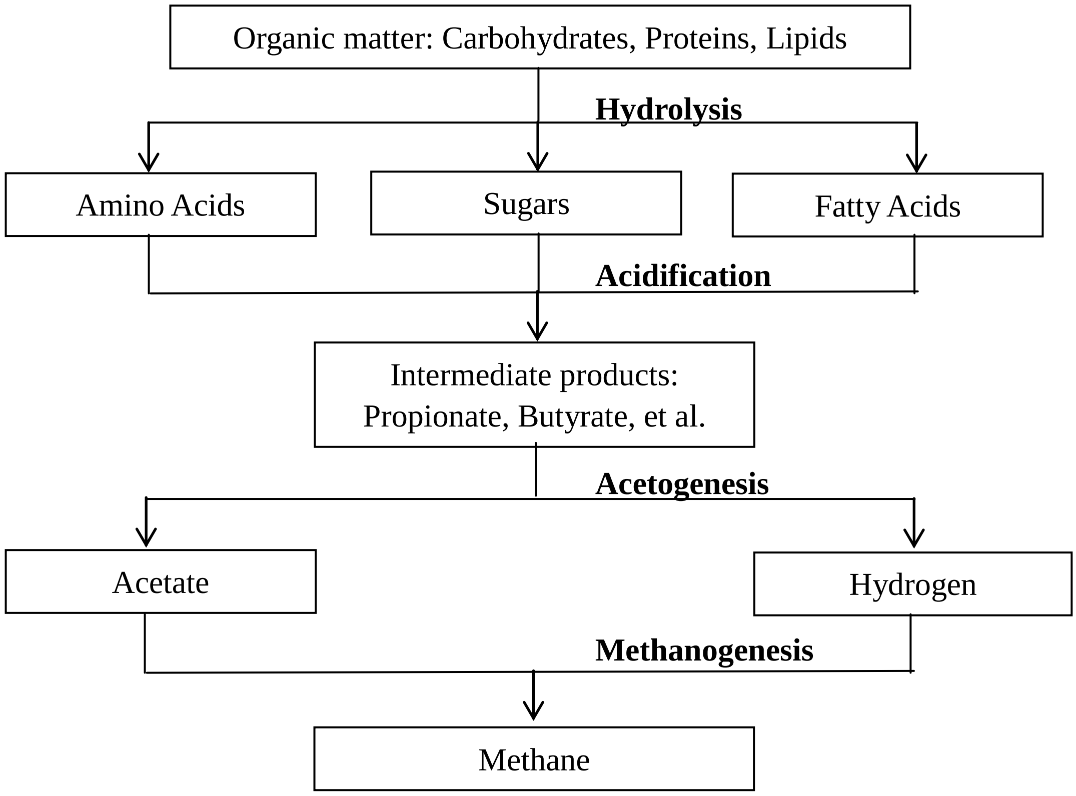


Figure 1.1 Schematic representation of the decomposition of swine wastes by anaerobic digestion

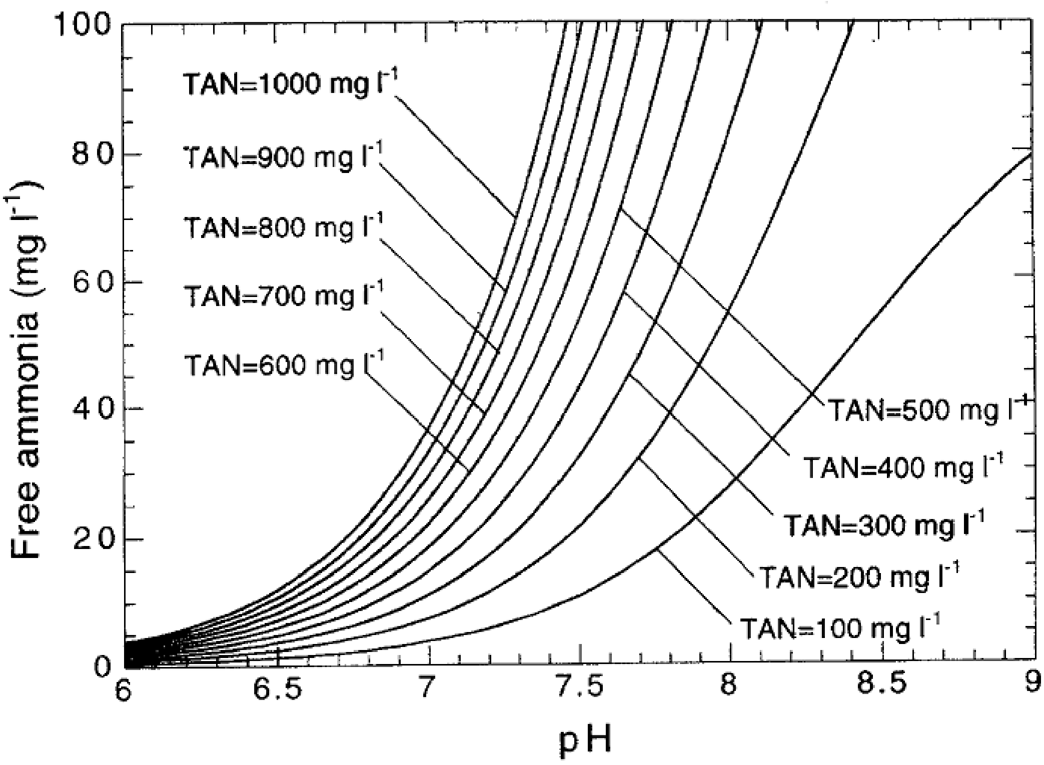


Figure 1.2 Free ammonia concentrations at various total ammonium nitrogen concentration and varying pH for a thermophilic digester [15].

Chapter 2 Development of a fixed zeolite bioreactor for anaerobic digestion of ammonium-rich swine wastes

2.1 Introduction

Anaerobic digestion is becoming increasingly attractive as a way to reduce the pollution caused by swine wastes. It effectively reduces organic matter and pathogenic microorganisms and exhibits the production of biogas, which helps to reduce conventional energy consumption [70]. In contrast to aerobic waste processes, anaerobic digestion does not require air input and the nutrient-rich digestate can be used as agricultural fertilizer. Moreover, produced methane is clean energy which could be an alternative to fossil fuel or petroleum for heat and electricity generation.

With the increasing demand of pork and accompanying environmental problems of swine wastes management, anaerobic digestion becomes a promising process due to its beneficial output including renewable energy production and mitigation of pollutant emissions in the closed system [71]. However, the digestion of animal dung (especially swine wastes or poultry manure) as the sole substrate has been unsuccessful because of ammonium inhibition which results in a low methane yield and a high VFA accumulation [72, 73]. Ammonium inhibition has occurred above pH 7.4 within the range of 1500-3000 mg NH_4^+-N/l during the anaerobic digestion process [74].

A high natural concentration of ammonium-nitrogen in manure and the produced ammonia as a common metabolic end product during the anaerobic digestion of protein-containing substrates is of high potential toxicity to methanogenic bacteria [75]. On the one hand, activities of methane synthesizing enzymes are directly inhibited by ammonia, on the other hand, ammonia molecules diffuse passively into the cell and are rapidly converted to ammonium which is toxic by altering the intracellular pH [76]. The methanogenic activity was reported to decrease by 10% at ammonium concentrations of 1670 to 3720 mg N/l, decrease by 50% at 4090 to 5550 mg N/l, and dropped to zero at 5880 to 6000 mg N/l [77]. Borja et al.[31] reported that an addition of ammonium from 2000 to 5000 mg N/l to the feed of thermophilic reactors result in a decrease in methane yield from 200 to 50 ml/g-VS. Therefore, it is necessary to manage the ammonium concentration in anaerobic digestion, so that the efficiency of biodegradation would be enhanced.

Many studies have been aimed to remove surplus NH_4^+ and improve digestion efficiency. Abouelenien et al. [78] developed a biogas recycling system for methane fermentation in nitrogen-rich chicken manure. Their system involved ammonia stripping coupled with ammonia fermentation using a rotary evaporator. At 55˚C and at an initial pH of 8-9, 195 ml/g-VS of methane was successfully produced from treated chicken manure. Nielsen and Angelidaki [72] have evaluated strategies for the recovery of ammonia-inhibited biogas via dilution. The results show that it is possible to improve the recovery rate for ammonia-inhibited anaerobic digester treating cattle manure by diluting the biomass with water or reactor effluent. Although the methods mentioned above are effective in improving methane production, the system is complicated, the volume of the reactor is large, and the construction and operation costs are high.

Some studies have shown that adding materials such as activated carbon, bentonite and zeolite into anaerobic digesters can significantly increase biogas production and organic matter degradation because these materials facilitate the adsorption of the related inhibitory compounds [79-81]. Adding such materials seems to be more practical and simple than the methods described above. Of the possible materials, zeolite is especially useful in improving the methane production of ammonium-rich biomass because it can facilitate the selective adsorption of ammonia and cation exchange in ammonium. In addition, the following characteristics also make zeolite promising for use in anaerobic digestion: (1) high capacity to immobilise microorganisms as a porous surface; (2) ability to withstand high temperature and acid; and (3) wide distribution, economic and obtainable [52]. The influence of zeolite concentration, zeolite dosage procedure, and zeolite type on the anaerobic digestion of ammonium-rich organic wastes has been researched by several authors. Tada et al. [53] have illustrated that natural mordenite has a synergistic effect on Ca^{2+} supply and NH_4^+ removal during the anaerobic digestion of ammonium-rich organic sludge. Montalvo et al. [54] have shown that adding natural zeolite on a daily basis is the most appropriate way to promote the continuous anaerobic digestion of swine wastes in terms of COD removal efficiency and methane production. Milán et al. [55] have suggested an optimum natural zeolite dosage of 2-4 g/l with an initial concentration of NH_4^+-N 410 mg/l. According to Kotsopoulos et al. [56], natural zeolite at doses of 8 and 12 g/l swine waste was better than doses of 0 and 4 g/l in terms of methane production in the context of thermophilic anaerobic digestion where the initial concentration of NH_4^+-N was less than 300 mg/l.

The results of these studies reveal that the addition of zeolite is effective in improving methane production and organic removal. However, all of these studies focus on an ordinary mixture of adsorption materials and fermentative feedstock; there is almost no research on the configuration of bioreactors using zeolite. Therefore, in this study, a fixed zeolite bioreactor was developed for use in the anaerobic digestion of ammonium-rich swine wastes. The reactor presents three advantages: (1) the zeolite can be conveniently replaced after maximum NH_4^+ adsorption; (2) the structure used is simple; and (3) the reactor is economical. Anaerobic digestion and methane production when the reactor was used were investigated.

2.2 Materials and methods

2.2.1 Seed sludge and swine manure

The anaerobic digestion seed sludge was collected in a digested sludge tank from a sewage treatment plant in Ibaraki prefecture, Japan (Table 2.1). The digested sludge was transferred into a plastic box and sealed, after which it was refrigerated under 4˚C. Before it could be used as inoculated sludge, 800 ml digested sludge and a synthetic medium containing yeast extract (300 mg/l), NH_4Cl (200 mg/l), KH_2PO_4 (16 mg/l), and a trace mineral solution (200 ml/l) were put into an anaerobic reactor (1000 ml), as in our previous work [82]. After one day, 1 g raw swine waste was added to this reactor every two days. The cultivation experiment was conducted at 35˚C for 10 days.

Because a higher concentration of ammonium-rich swine waste was required, the raw swine waste used in the experiment was stale manure that had been kept at room temperature for almost two years after it had been obtained from a swine farm located in Tokyo. Compared with fresh swine waste, stale manure has a higher concentration of ammonium; in fact, it can reach levels of up to 22310 mg/l. After the swine waste was diluted with tap water and its pH adjusted with HCl, it was inoculated with 20% sludge (w/w). The characteristics of the diluted swine waste used for methane fermentation are shown in Table 3.1.

2.2.2 Adsorption experiment

The synthetic zeolite (zeolite A-3) used as the adsorbent in the experiments was obtained from Wako Pure Chemical Industries, Ltd. The characteristics of zeolite A-3 are shown in Table 2.2.

To investigate the adsorption capacity of zeolite A-3, batch adsorption isotherm tests were performed for ammonium removal. 20 g/l zeolite A-3 was introduced into five 50 ml centrifuge tubes, to which 40 ml of ammonium chloride solution with a concentration between 3000 and 7000 mg NH_4^+-N/l was added. The centrifuge tubes were heated in a water bath at 35˚C for 24 hours. The final concentration of NH_4^+-N remaining in the solution was analysed, and the amount of ammonium adsorbed was calculated using the following equation:

$$G = (C_0 - C_{eq})V/m \quad (1)$$

where G is the amount of ammonium adsorbed per unit weight of zeolite (mg/g), C_0 is the initial concentration of NH_4^+-N (mg/l), C_{eq} is the concentration of NH_4^+-N at equilibrium time (mg/l), V is the solution volume (l), and m is the absorbent dosage (g).

2.2.3 Anaerobic digestion experiment

To investigate the performance of methane fermentation in different bioreactor configurations, three groups of experiments were performed in three different bioreactors: a fixed zeolite bioreactor, a sunken zeolite bioreactor and a bioreactor without zeolite. The experiments were conducted in batch mode at 35˚C for 32 days (Fig.2.1). Fermentor bottles (300 ml, SIBATA) were used as bioreactors. Each bioreactor contained 200 ml of diluted swine waste including 20% (w/w) digested sludge. In the fixed zeolite bioreactor, 4 g zeolite A-3 was fixed in a porous nylon bag (pore diameter = 3 mm, volume = 12 ml) suspended in the diluted swine waste prepared as indicated in *2.1*. For sunken zeolite bioreactor, the same dosage of zeolite was dispersed at the bottom of the reactor. Each group of experiments was performed in duplicate.

2.2.4 Analytical methods

The yield and composition of the biogas produced were determined every day. The biogas was collected using two 50-ml plastic syringes, and the volume was read directly using the scale on the syringe. The gas composition was detected via gas chromatography (GC-8A, SHIMAZU, Japan) using a machine equipped with a thermal conductivity detector (80˚C) and a Porapak Q column (60˚C). Nitrogen was used as the carrier gas. COD, TS, VS, and TN were detected in accordance with standard methods, and pH was determined using a pH meter (TES 1380) and test paper (pH 6.4-8.0). The amount of ammonium nitrogen was determined using an ion meter (Ti 9001, Toyo Chemical Laboratories Co., Ltd.). The activity of the microorganisms was indicated by the ATP concentration, which itself was determined using a Bac Titer-Glo™ Microbial Cell Viability Assay (Promega, USA).

2.3 Results and discussion

2.3.1 Adsorption isotherm analysis

It is well known that ammonium ions are removed from aqueous solutions by zeolite when they are exchanged with cations or via adsorption into the pores of aluminosilicate systems [83]. The Freundlich model is frequently used to describe equilibrium adsorption data, which are established when the concentration of the adsorbate in the bulk solution is in dynamic balance with that on the liquid-solid interface [84, 85]. Therefore, in this study, the Freundlich model was employed to evaluate the distribution of ammonium between the zeolite phase and solution phase and is expressed mathematically as follows:

$$\mathrm{G} = \mathrm{K_f} \mathrm{C_{eq}}^{1/\mathrm{n}} \tag{2}$$

The linear form of Eq. (2) is:

$$\mathrm{logG} = \mathrm{logK_f} + \frac{1}{\mathrm{n}} \mathrm{logC_{eq}} \tag{3}$$

where K_f and *n* are the Freundlich coefficients. K_f indicates the adsorption capacity (mg/g), and *n* is related to the intensity of the Freundlich isotherm. Plotting the experimental data using equation (14) indicates that the model fits the data well (R^2 = 0.985). As a result, the Freundlich isotherm for ammonium removal on the zeolite A-3 can be represented by equation (4):

$$G = 1.13C_{eq}^{0.53} \tag{4}$$

The Freundlich constant *n* values (1.90) indicate the high strength of the bond between the adsorbate and adsorbent; the surface of the zeolite is hydrophilic and attracts ammonium ions in aqueous solution [86]. Therefore, as an absorbent, zeolite A-3 could be an excellent material to use to remove ammonium from ammonium-rich swine waste.

2.3.2 The performance of anaerobic digestion experiment

Three different bioreactors were fed with the adjusted swine wastes with an initial ammonium nitrogen concentration of 3770 mg/l. The optimum zeolite dosage was 5%-10% (w/w organic waste) under an NH_4^+-N concentration of 4500 mg/l [53]. Therefore, in this study, 20 g/l (almost 5% w/w swine wastes) of zeolite was used in the fixed zeolite bioreactor. Biogas production in the fixed zeolite bioreactor was compared with biogas production in the bioreactor with sunken zeolite at the bottom and in the one without zeolite.

Fig. 2.2 A indicates that the startup period for anaerobic digestion was 14 days in the fixed zeolite bioreactor. Beginning on the 14th day, biogas production increased gradually to the daily maximum of 775 ml/l on the 24th day, and the corresponding methane concentration increased from 62.5% to 82.2% (Fig.3.2 B). Thereafter, the daily methane yield decreased day by day, but the methane concentration remained at around 80% until the end of the batch experiment. The fixed zeolite bioreactor exhibited better performance than two other bioreactors in terms of biogas production and methane concentration (Fig. 2.2 A, B). It can be concluded that the others cause longer startup time (20 days) and lower daily methane yield. Although there was no obvious difference between the three systems in terms of methane concentration during the first 14 days, the concentration of methane in the fixed zeolite bioreactor was always higher than that in the other two from day 15 to day 32.

These results demonstrate that anaerobic digestion in the fixed zeolite bioreactor is effective in improving methane production. Because ammonium, which inhibits the microorganisms, was partially be removed by the zeolite, the NH_4^+-N level was 2620 mg/l on day 3 (Fig.2.2 C). However, this value was almost 3000 mg/l in the bioreactor with the sunken zeolite. This means that the fixed zeolite bioreactor is more effective in removing ammonium than the sunken zeolite bioreactor. The reason may be that in the former case, the zeolite was suspended in the upper layer of the digested liquid, where ammonium could easily be trapped by the adsorbent. When zeolite was dropped into the bottom of the reactor, it would have been difficult for it to make contact with the ammonium in the liquid because the swine waste would have covered the surface of the adsorbents. The ammonium concentration increased during the anaerobic digestion process because ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of proteins and urea [87]. After the startup period, the ammonium concentration increased gradually. After 32 days, the total NH_4^+-N concentration in the fixed zeolite bioreactor, the bioreactor with sunken zeolite at the bottom, and the bioreactor without zeolite had increased to 3974, 3558, 4580 mg/l, respectively (Fig.2.2 C).

Overall, the pH value in the three reactors was between 7.0 and 8.0 and thus fulfilled the conditions for anaerobic digestion (Fig.2.2 D). In the fixed zeolite bioreactor, the pH decreased slightly (from 7.2 to 7.0) during the startup period, perhaps because of the production of volatile fatty acid (VFA). Beginning on day 15, the pH progressively increased to 8.0; then, it remained at 8.0 until the end of the digestion process. The increase in pH can be explained by the increase of ammonium concentration and the biodegradation of VFA into methane gas. The pH in other two bioreactors remained constant until day 25 and then increased to 7.6 on day 32. This pattern is consistent with the result of biogas production.

2.3.3 Microorganism activity

The quantity and activity of the microorganisms in a bioreactor are two critical parameters that determine the reactor's performance [88]. ATP is an indicator of metabolically active cells and an index of microbial density, which has been shown to reflect the activity of anaerobic digestion [89, 90]. In this study, ATP concentration was tested on the surface of the zeolite in the fixed zeolite and sunken zeolite bioreactors, and in the liquid from the three bioreactors at the end of the digestion experiment (Fig. 2.3). It is clear that the ATP value is similar in the liquid phase for the three bioreactors but that the concentration on the surface of the zeolite in the fixed zeolite bioreactor is much higher. This indicates the high activity levels of the immobilised microorganisms on the surface of the fixed zeolite, which could be interpreted as indicating that the fixed zeolite is a stable and suitable carrier for microbes and that most microorganisms grew on the surface of the fixed zeolite. The large quantities of microbes would have also contributed to the high concentration of ATP. The distribution of microbes in the liquid phase and on the surface of the support materials were about 5% and 95% respectively [91]. In contrast, ATP concentration is low on the surface of the sunken zeolite. This is because the sunken zeolite in the bioreactor is mobile and unstable; the microorganisms could have easily been washed out by sampling or shaking, making this an unsuitable environment for microbes to grow in. Therefore, the fixed zeolite bioreactor showed the best performance with regard to the anaerobic digestion of ammonium-rich swine wastes.

2.3.4 Effectiveness of fixed zeolite bioreactor with regard to the digestion of ammonium-rich swine wastes

Fig.2.4 shows total methane production and COD removal efficiency for the three batch experiments. In this study, the methane production and COD removal figures are 178.5 ml/g-VS and 46.6%, respectively, for the fixed zeolite bioreactor. These figures are lower than the theoretical methane production value of 516 ml/g-VS for swine wastes [92]. However, whereas the theoretical value is based on the assumption that all of the carbon substrate transformed into methane gas, a fraction of the substrate is in fact used to synthesise bacterial mass [93]. Moreover, the relatively high initial concentrations of NH_4^+-N and COD are another factor that contributed to the lower actual methane yield.

Sánchez et al. [70] investigated pig waste treatment using an upflow anaerobic sludge bed reactor (UASB) and an anaerobic fixed bed reactor (AFBR) at COD and

NH_4^+-N concentrations less than 12600 mg/l and 650 mg/l, respectively. Their study yielded COD removal efficiency rates of 60% and 40% for the AFBR and UASB reactors, respectively. Here, however, 46.6% of COD was removed at the higher initial COD concentration of 41000 mg/l and the higher initial NH_4^+-N concentration of 3770 mg/l. In addition, the methane production level of 178.5 ml/g-VS achieved using the fixed zeolite bioreactor is much better than that achieved using the control, which was 57.6 ml/g-VS under the same operating conditions. The fixed zeolite bioreactor is effective in improving the methane production of ammonium-rich swine wastes.

Furthermore, the ammonium adsorption capacity of zeolite A-3 is almost 50 mg NH_4^+-N/g-zeolite in the present study. This means that 5% of the ammonium nitrogen was adsorbed in zeolite. If the ammonium saturated zeolite is used as fertiliser directly, such as for soilless culture, the annual production of nitrogen fertiliser can be decreased, and environmental pollution can thereby be reduced by recycling nitrogen resource, and save fertilizer cost $.13 per acre-inch (Lambert, 1990).

Further investigations of continuous anaerobic digestion should be conducted to determine the practical effectiveness of this technique. We predict that, in the continuous reaction, when the concentration of ammonium is up to 3000-3500 mg/l (almost after15-20 days), the ammonium saturated zeolite can be replaced by new one. In this way, the ammonium can be removed again, and the continuous anaerobic digestion system could be stable and sustainable.

2.4 Summary

A fixed zeolite bioreactor was developed for use in the anaerobic digestion of ammonium-rich swine wastes. It can be concluded from the results that (1) the pattern of ammonium adsorption of zeolite A-3 followed the Freundlich isotherm equation; (2) the start-up time decreased (and methane production increased) when the fixed zeolite bioreactor was used; and (3) the immobilised microorganisms on the surface of the fixed zeolite facilitated effective anaerobic digestion and endured to high ammonium inhibition.

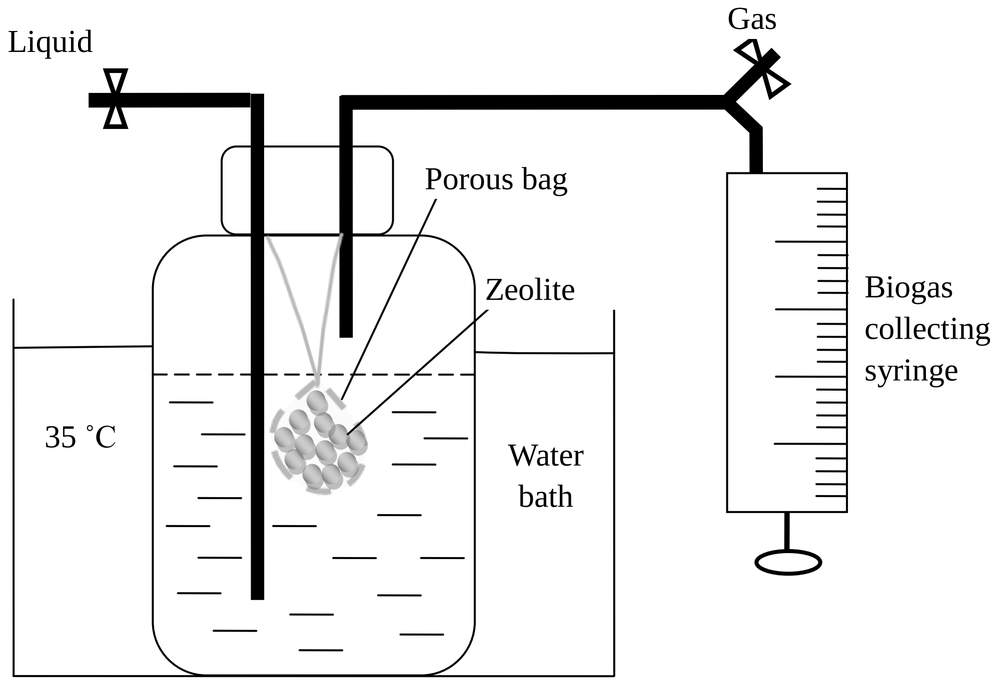


Figure 2.1 Schematic diagram of the fixed zeolite bioreactor.

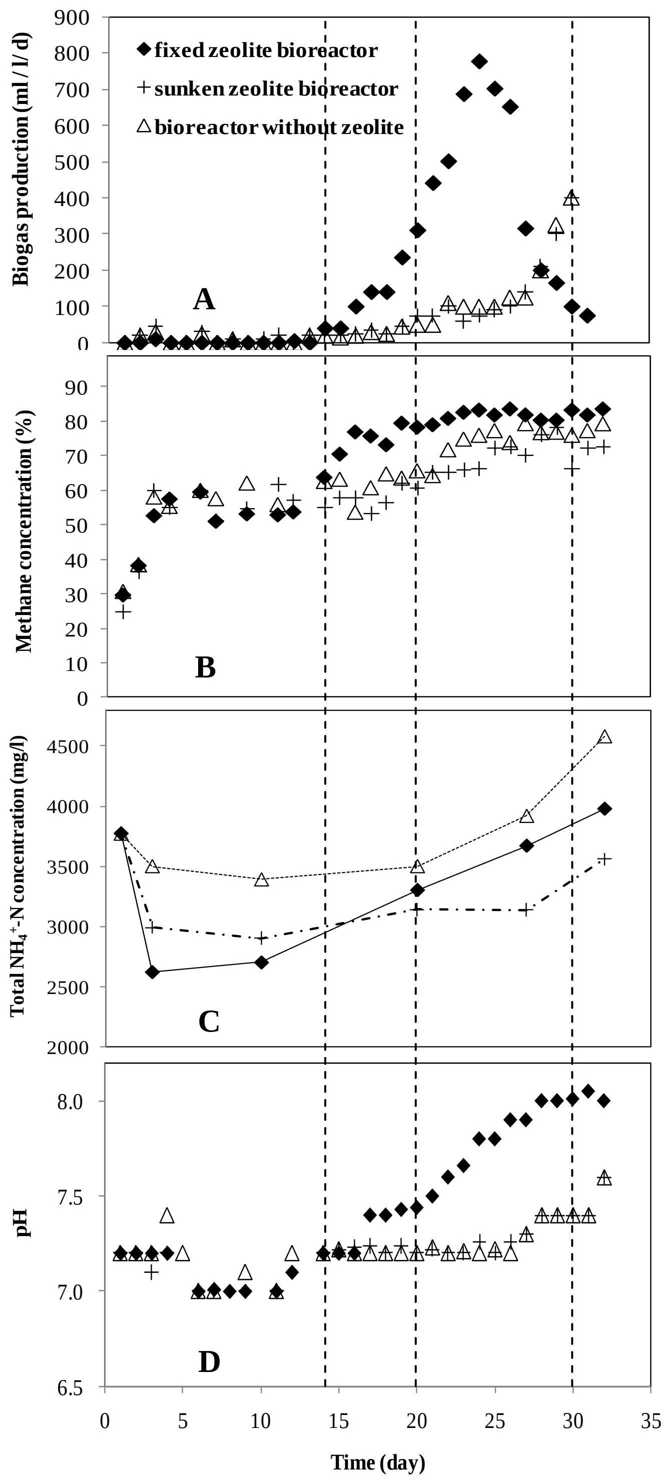


Figure 2.2 The performance of the three bioreactors for the anaerobic digestion of swine wastes during the experiment. methane production (A); methane concentration (B); pH value (C); ammonium nitrogen concentration (D).

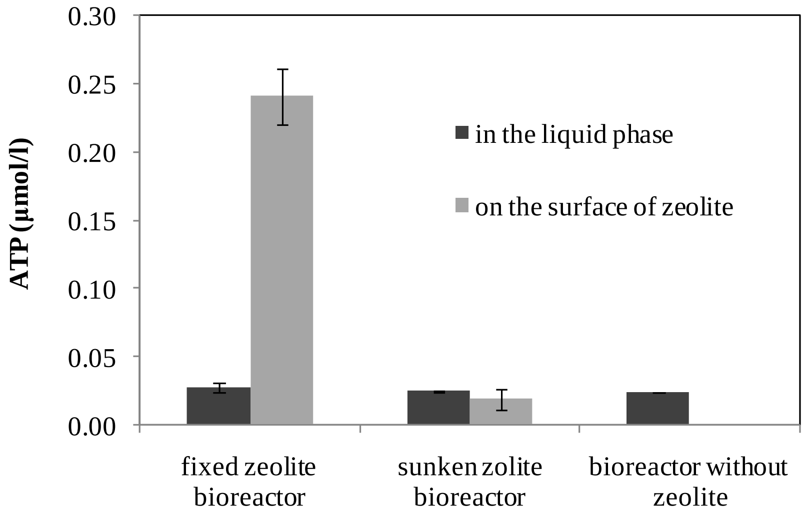


Figure 2.3 ATP values for the bioreactors at the end of the experiment. The bars designate standard deviations (95% confidence, t-test).

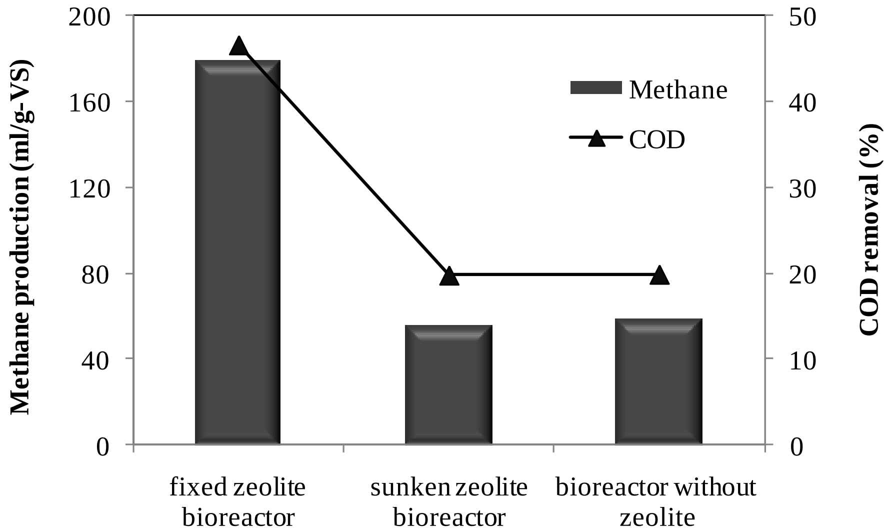


Figure 2.4 Methane production and COD removal in the three bioreactors.

Table 2.1 The characteristics of seed sludge and swine waste used in the experiments after dilution with deionised water and with adjusted pH

Parameters	Digestion sludge	Swine wastes
COD (mg/l)	6500	41000
TN (mg/l)	5489	39402
TS (mg/l)	13292	33900
VS (mg/l)	9500	27150
NH_4^+-N (mg/l)	1547	3770
pH	7.1	7.2

Table 2.2 The characteristics of zeolite A-3 (Wako company supply)

Pore diameter (Å)	Absorbable molecule	Unabsorbable molecule	Water absorbing capacity (wt%)	General formula	Particle size (mm)
3	H_2O,NH_3 ,He diameter <3A	CH_4,CO_2,C_2H_2,O_2,H_2S, C_2H_5OH diameter>3A	20	$(0.4K+0.6Na)_2O \cdot Al_2O_3 \cdot 2SiO_2$	2.36−4.75

Chapter 3 Development of a modified porphyritic andesite as ammonium adsorbent and bed material

3.1 Introduction

From these results of chapter 2, it is clear that the zeolite fixed bioreactor is effective in partially removing ammonium to improve the anaerobic digestion of ammonium-rich swine waste. However, the adsorbent of zeolite A-3 is from Woko Company which is a kind of expensive synthetic. Moreover, to overcome the ammonium inhibition in the anaerobic digestion system, both of the ammonium removal and bioactivity improvements need to be considered. Therefore, materials with both bioactivity and ammonium adsorption capacity need to be developed to fulfill this research gap.

Porphyritic andesite is a material that is commercially known as wheat-rice-stone (WRS). WRS has been widely used in recent years because it is an effective adsorbent in environmental and health-based applications such as water purification, odor and taste adsorption and medical treatments [94]. It has also been utilized in many industrial and agricultural applications, such as paper manufacturing, soil improvement and catalyst scaffolding, and as an additive in products such as fertilizer [95]. Its utility in these applications can be ascribed to its structure as a clay mineral and its bioactivity, absorbability and stability in a wide pH range [96]. The accessibility and abundance of WRS in East Asia make it an attractive material for use in the anaerobic digestion processes. In recent years, WRS has been applied in anaerobic digestion of solid organic wastes for volatile organic acid adsorption and cation dissociation [97]. However, there have been few studies on the use of WRS for ammonium removal in the anaerobic digestion of ammonium-rich organic wastes.

Therefore, the objectives of this study were to develop a Ca-modified WRS with bioactivity and an enhanced ammonium adsorption capacity for the anaerobic digestion of ammonium-rich organic wastes, to evaluate the characteristics of Ca-modified WRS by SEM and BET analyses, and to describe the adsorption process by adsorption kinetics and isotherm models. We also investigated its performance in the removal of ammonium during the anaerobic digestion of ammonium-rich swine waste.

3.2 Materials and methods

3.2.1 Generation and characterization of Ca-modified WRS

The natural WRS used in this study was obtained from Henan province, China. Its chemical composition is listed in Table 3.1. Integrated Ca-salt treatment and calcination methods were developed to modify the natural WRS. First, we ground natural WRS and filtered it through a sieve to obtain samples with diameters less than 0.3 mm. Next, the WRS powder was mixed with bentonite, starch and calcium salt to improve its hardness, porosity and ion exchange capacity. Then, different types of calcium salt ($CaCl_2$, $CaSO_4$, and $CaCO_3$) were added at various concentrations (5%, 10% and 15%) investigate the conditions for optimum ammonium uptake. Ultra-pure water was then added into the mixture to make a paste; the granulation procedure was performed manually. After that, the granules were dried at 105 ℃ for 24 h and calcined in a muffle furnace for 2 h at different temperatures (500, 600, and 700 ℃). Finally, the Ca-modified WRS was cooled to room temperature for further use.

3.2.2 Batch adsorption of ammonium

A stock solution of 6000 mg N/l ammonium was prepared from anhydrous NH_4Cl and was diluted to the desired concentrations using distilled water. All of the adsorption experiments were performed with an adsorbent dose of 20 g/l WRS in 50 ml centrifuge tubes, which were immersed in a temperature controlled water bath. The effect of contact time (0-48 h) on ammonium uptake was examined at 35 ℃ with an initial ammonium concentration of 5000 mg N/l. The kinetics of ammonium adsorption was determined by analyzing the uptake of ammonium from aqueous solutions by the modified WRS at different time intervals. The adsorption isotherm was determined by varying the initial ammonium concentration from 2000 to 6000 mg N/l at 35 ℃. The effect of pH was investigated by adjusting the pH from 5 to 9 with 0.1 M HCl and 0.1 M NaOH from an initial ammonium concentration of 5000 mg N/l.

3.2.3 Analytical methods

The specific surface areas of the natural and modified WRS were determined by the BET method with N_2 gas in a gas adsorption analyzer (Coulter SA3100, Japan). The morphological images were acquired with a scanning electron microscope (SEM)

(JSM-6700F, JEOL, Japan). The amount of ammonium nitrogen was determined using an ion meter (Ti 9001, Toyo Chemical Laboratories Co., Ltd.) and pH was determined using a pH meter (TES 1380)

All chemical reagents, including the wheat starch (granule size: 1–5μm) and calcium salt, used in this study were analytical grade (Wako Pure Chemical Industries, Japan), and all stock solutions were prepared in ultra-pure water (resistivity 18.2MΩ.cm at 25°C) prepared with a water purification system (Purelite PRB-001A/002A) supplied by Organo, Japan.

3.3 Results and discussion

3.3.1 The investigation of calcium salt types and their effective doses for WRS modification

Modification by salt treatment improves the ability of materials to adsorb ammonium by significantly enhancing their ammonium ion exchange capabilities. The ion exchange reaction is a stoichiometric process, where one equivalent of an ion in the solid phase is replaced by an equivalent ion from a solution. Cations that exchange ammonium (Na^+, K^+, Ca^{2+} and Mg^{2+}), especially Na^+ and Ca^{2+}, are widely used in modification processes [98, 99]. Calcium has a positive effect on the anaerobic digestion of organic wastes [100]. Ahn et al. [101] reported that methane production was improved by increasing the concentration of calcium in an anaerobic digestion reaction. Therefore, we treated natural WRS with calcium salt. The ion exchange reaction can be written as the following:

$$Ca^{2+}(S) + 2NH_4^+(L) \leftrightarrow 2NH_4^+(S) + Ca^{2+}(L) \quad (1)$$

where S and L refer to the solid and liquid phases, respectively. Reaction (1) shows that ammonium in the solution is removed while calcium is released. Thus, Ca-modified WRS is suitable for ammonium removal and calcium dissociation in anaerobic digestion bioreactors.

Fig. 3.1 shows the ammonium adsorption capacity of WRS modified with $CaCl_2$, $CaSO_4$, or $CaCO_3$. Different types of Ca-salt modifications result in different ammonium adsorption capacities. The ammonium adsorption capacity of $CaCl_2$-modified WRS was much higher than WRS modified with other calcium salts, so $CaCl_2$ was used in the modification process for subsequent studies. Afterward, the optimum concentration of $CaCl_2$ was determined. Fig. 3.2 shows the ammonium adsorption capacity of Ca-modified WRS at $CaCl_2$ concentrations of 5%, 10% and 15%. The ammonium adsorption capacity with a $CaCl_2$ concentration of 5% is 20.3 mg N/g, which is much lower than the adsorption capacities at higher concentrations. The ammonium adsorption performance is similar with $CaCl_2$ concentrations of 10% and 15%. Therefore, from an economic point of view, the optimum $CaCl_2$ concentration is 10%, where the ammonium adsorption capacity is 36.5 mg N/g at an initial ammonium concentration of 5000 mg N/l. In addition, we used a concentration of 10% for both bentonite and starch; the sturdiness and durability of the modified material could not be achieved at lower or higher concentrations (data not shown).

3.3.2 The investigation of the calcination temperature

Calcination temperature is related to the structure of the adsorbent material as well as energy consumption. Low temperatures lead to the reduced consolidation of the adsorbent granule, while high temperatures not only destroy pore channels of the adsorbent but also increase energy consumption. Consequently, the optimum calcination temperature of the Ca-modified WRS was investigated. Fig. 3.3 shows the ammonium adsorption capacity performances of natural WRS and WRS modified at different calcination temperatures. Compared with natural WRS, the ammonium adsorption capacity of Ca-modified WRS was much higher. A calcination temperature of 600 ℃ resulted in a better performance than both 500 ℃ and 700 ℃. In addition, the Ca-modified WRS calcined at 600 ℃ is sturdy and durable enough to maintain its granular shape even after immersing it in the ammonium solution for 30 days.

3.3.3 Characterization of modified WRS

The modified WRS is a gray-black color and is 2-3 mm in diameter. The SEM photographs in Fig. 3.4 were taken at 1000x magnification to observe the surface morphology of the natural and Ca-modified WRS. The surface of the natural WRS is smooth, and few pores were observed. Compared with the natural WRS, the surface of the Ca-modified WRS is rougher and pores diameters are larger, which indicates that a porous texture with irregularly defined channels was formed. Calcination can decompose organic matter, remove water from the surface of a material and enlarge pore diameter [102]; these changes could improve material reactivity and impart a higher adsorption capacity. In this study, a portion of the pore-forming material (starch) was oxidized to CO_2 at 600 ℃, causing pores to be formed when the CO_2 gas escaped during the calcination processes.

The specific surface area and total volume of the Ca-modified and natural WRS, listed in Table 3.2, further confirmed the above results. The specific surface area of the Ca-modified WRS is 4.56 sq. m/g, and the pore volume is 0.021 ml/g. These parameters for the natural WRS are 0.97 sq. m/g and 0.003 ml/g, respectively. We demonstrated that the adsorption capacity of the natural WRS was improved through the modification processes. The pore size distributions of the Ca-modified WRS are given in Fig. 3.5, which shows that the observed pore sizes mostly varied between 6 and 80 nm (64.73%). The radius of ammonium (1.47 Å) is much smaller than this range (6–80 nm), suggesting an improvement in the probability of ammonium ions dispersing into the inner layer of the granular adsorbents.

3.3.4 Adsorption isotherm models

Adsorption isotherms help establish the adsorption mechanism for the interaction of the adsorbate with the adsorbent. The distribution of ammonium between the liquid and solid phases in the adsorption process can be expressed by the Freundlich and Langmuir isotherm models.

The Freundlich equation models multilayer adsorption and the adsorption by heterogeneous surfaces [103]. The linearized form is as follows:

$$\log q_e = \log K_f + n_f \log C_e \quad (2)$$

where C_e is equilibrium concentration, q_e is equilibrium adsorption capacity, and K_f and n_f are Freundlich constants related to the minimum adsorption capacity and the adsorption intensity, respectively. The values of K_f and n_f were obtained from the slope and the intercept of the linear Freundlich plot of log q_e versus log C_e.

The Langmuir adsorption isotherm models the monolayer coverage of the adsorption surfaces and assumes that adsorption take places on a structurally homogeneous surface of the adsorbent [104]. The linear form of the Langmuir model can be presented as the following:

$$1/q_e = 1/bQ_mC_e + 1/Q_m \tag{3}$$

where C_e and q_e are the concentration of ammonium and ammonium adsorption capacity, respectively, at equilibrium. Q_m is the maximum adsorption capacity, and b is the Langmuir adsorption constant related to the affinity of the binding sites and the energy of the adsorption. The values of the Langmuir parameters, Q_m and b, were calculated from the slope and the intercept of the linear plots of $1/q_e$ versus $1/C_e$.

The essential features of Langmiur isotherm can be expressed in terms of dimensionless equilibrium parameter R_L that is given by the following equation:

$$R_L = 1/(1+bC_0) \tag{4}$$

where C_0 is the initial amount of adsorbate. R_L value between 0 and 1 represents suitability of the adsorbent for the adsorbate [105].

The coefficients calculated by experimental data and Eqs. (2) and (3) are shown in Table 3.3. The results show that for natural and Ca-modified WRS, the two models are suitable (R^2>0.95). However, the Freundlich model provides a slightly more consistent fit compared with the Langmuir model. It has been suggested that heterogeneous uptake of ammonium occurs on both natural and Ca-modified WRS. The equilibrium pattern is similar to one in Lei's [106] report, in which microwave-treated natural Chinese zeolite was used to adsorb ammonium. In the Langmuir equation, we obtained a Q_m coefficient of 45.45 mg N/g for Ca-modified

WRS and 23.98 mg N/g for natural WRS. In the Freundlich equation, we obtained K_f coefficients of 0.130 mg N/g and 0.001 mg N/g for Ca-modified WRS and natural WRS, respectively. The Q_m and K_f values represent the ammonium amount that can be adsorbed. Therefore, our results indicate that the Ca-modified WRS has a higher adsorption capacity than natural WRS. The constant n_f is a measure of adsorption intensity or surface heterogeneity and ranges between 0 and 1 [107]. In this study, the n_f constant for the Ca-modified WRS is less than unity, which indicates that the adsorption condition is favorable. Moreover, the calculated Langmuir equilibrium parameter R_L of the modified WRS is 0.07 ($0< R_L<1$) suggesting that the uptake process is a favourable one. Therefore, the modified WRS could be an excellent adsorbent to remove ammonium from ammonium-rich swine waste.

3.3.5 Adsorption kinetics

The study of adsorption kinetics provides valuable insights into the reaction pathway and describes the solute uptake rate, which in turn controls the amount of time the adsorbate resides at the solid-solution interface [105]. To investigate the ammonium uptake process by the Ca-modified WRS, the effect of contact time was assessed over a period 48 h at an initial NH_4^+-N concentration of 5000 mg/l. According to the kinetic data obtained from the experiments, Lagergren first-order and Ho's pseudo-second-order kinetic models can be used to elucidate the mechanisms of adsorption [108, 109]. These equations can be written as the following:

$$\ln (q_e - q_t) = \ln q_e - k_1 t \quad (4)$$

$$t/q_t = 1/k_2 q_e^2 + t/q_e \quad (5)$$

where q_t and q_e represent the amount of adsorbed ammonium at time t and at the equilibrium time, respectively. k_1 and k_2 are, respectively, the-first order and second-order rate constants for adsorption. k_1 is determined from the slope of the linear plot of log (q_e−q_t) versus t, and k_2 is obtained by plotting t/q_t versus t.

The adsorption kinetic constants obtained from the two models are listed in Table 3.4. The results show that, for both Ca-modified and natural WRS, the correlation coefficient values (R^2=0.999, 0.999) for Ho's pseudo-second-order model are much higher than those obtained from first-order kinetics (R^2=0.415, 0.688). Thus, a pseudo-second-order model explains the kinetic processes better. The predicted value of $q_{e,}$ using the pseudo-second-order model is 35.84 mg N/g for Ca-modified WRS and 8.35 mg N/g for natural WRS, which are very close to the values obtained from our experimental data (36.60 mg N/g for Ca-modified WRS and 9.52 mg/g for natural WRS). From the values of the second-order rate constant, k_2, the ammonium uptake rate by Ca-modified WRS is much higher than that of natural WRS. The kinetic data indicate that ammonium adsorption by Ca-modified WRS obeys pseudo-second-order kinetics, which suggests a chemisorption process in this experiment.

3.3.6 The effect of pH on the ammonium adsorption capacity of Ca-modified WRS

Ammoniacal nitrogen is present in aqueous solution in two forms: free ammonia (NH_3) or ionised ammonium (NH_4^+). The equilibrium between ammonia and ammonium in solution is dependent on pH [110]. The adsorption capacity is related to the existing form of ammoniacal nitrogen. Therefore, to analyze the effect of pH on ammonium uptake by Ca-modified WRS, the ammonium adsorption capacity was determined at a pH range from 5.0 to 9.0. Our results show that the ammonium adsorption capacity remained almost constant over the pH range of 5.0-9.0, with a value of 36.5 mg N/g. This suggests that Ca-modified WRS not only performs well to adsorb ammonium in neutral solutions but also in weakly acidic and weakly alkaline conditions. This result is similar to Li's [99] report, where modified zeolite was used to remove ammonium from drinking water. In practice, the pH varies between 6.0 and 8.5 in an anaerobic digestion bioreactor. Considering its successful performance in ammonium uptake over a pH interval of 5.0-9.0, Ca-modified WRS is an appropriate material for practical use.

3.4 Summary

We developed a Ca-modified WRS to improve the ammonium adsorption capacity of natural WRS. We determined that the specific surface area and total pore volume of the Ca-modified WRS were 4.56 sq. m/g and 0.021 ml/g, which indicate improvements over natural WRS. The adsorption of ammonium by Ca-modified WRS can be described by Freundlich and Langmuir isotherm models, and the maximum adsorption capacity is 45.45 mg N/g. The kinetics results indicate that ammonium adsorption by Ca-modified WRS followed a pseudo-second-order kinetic model. The ammonium adsorption capacity remained constant at a pH range between 5.0 and 9.0, which implies that Ca-modified WRS is a suitable adsorbent for practical applications. Based on its excellent performance in anaerobic digestion experiments, Ca-modified WRS can be an effective material for use in improving the anaerobic digestion of ammonium-rich wastes.

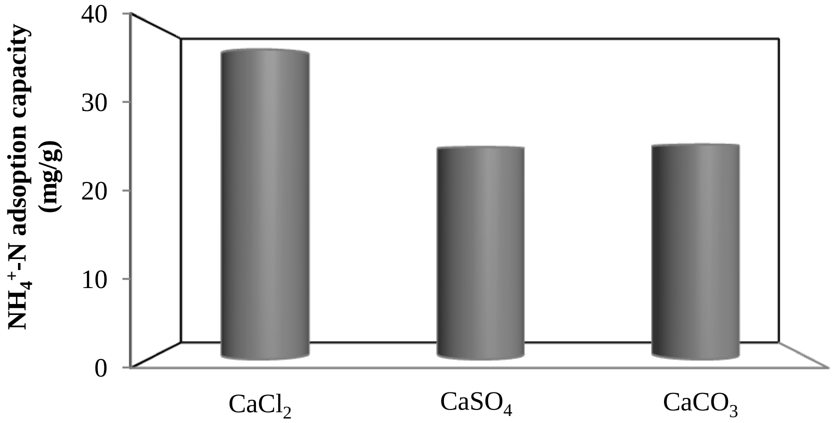


Figure 3.1 Ammonium adsorption capacity of WRS modified with different calcium salt (initial NH_4^+-N = 5000 mg/l, adsorbent dose = 20 g/l, pH = 7.0)

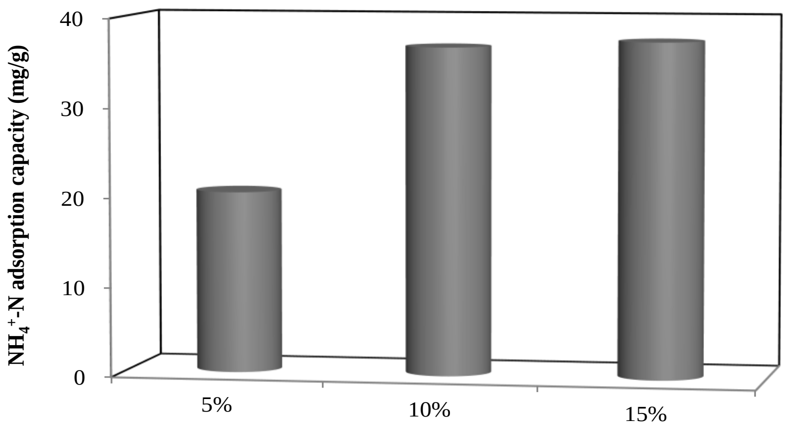


Figure 3.2 Ammonium adsorption capacity of Ca-modified WRS at different concentrations of $CaCl_2$ (initial NH_4^+-N = 5000 mg/l, adsorbent dose = 20 g/l, pH = 7.0)

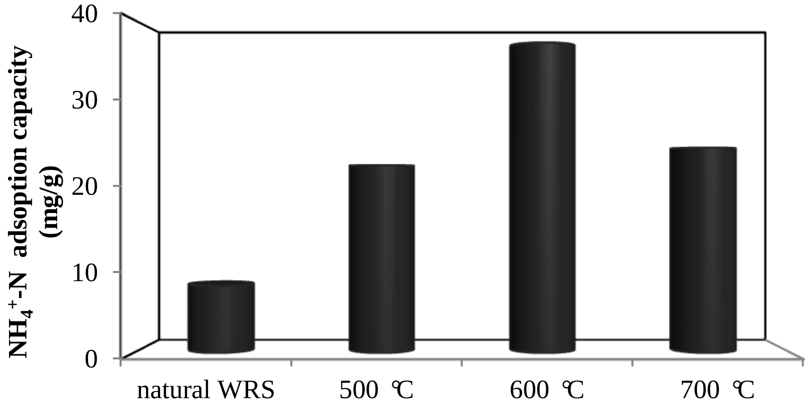


Figure 3.3 Ammonium adsorption capacity performance of natural WRS and WRS modified at different calcination temperatures (initial NH_4^+-N = 5000 mg/l, adsorbent dose = 20 g/l, pH = 7.0)

(a)

(b)

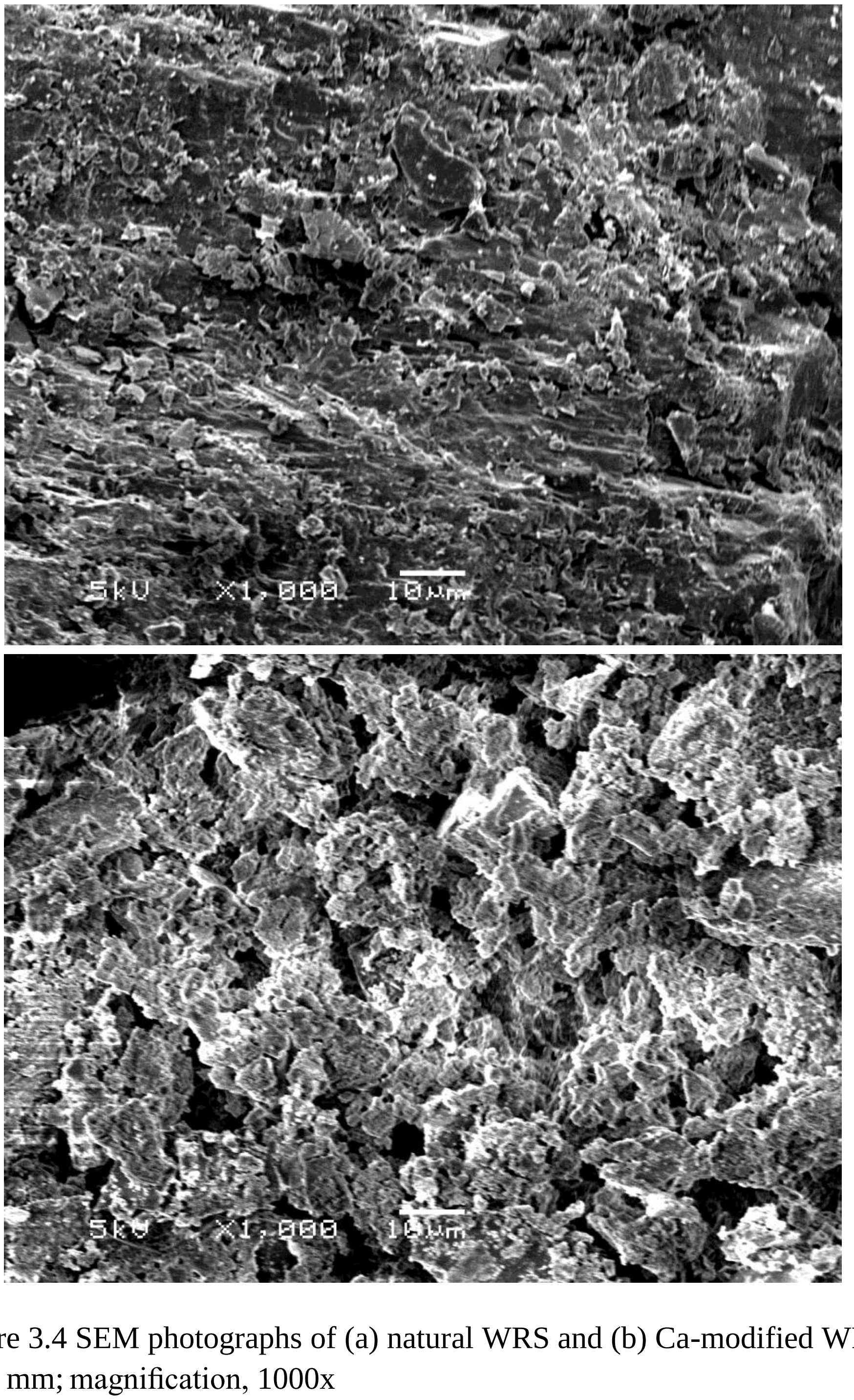


Figure 3.4 SEM photographs of (a) natural WRS and (b) Ca-modified WRS. Scale bar is 10 mm; magnification, 1000x

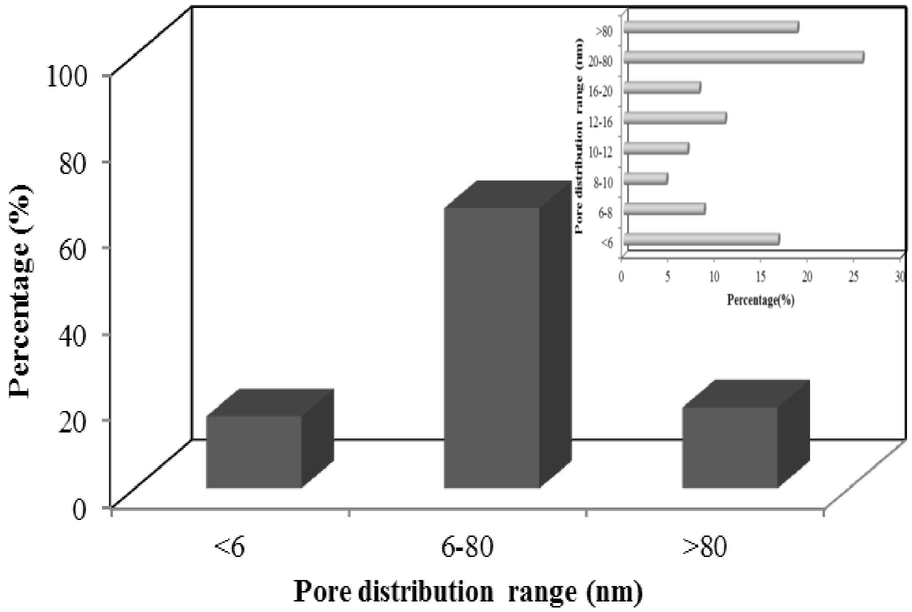


Figure 3.5 BJH (Barrett-Joyner-Haloenda) pore-size distribution of Ca-modified WRS

Table 3.1 Chemical composition of the porphyritic andesite

Component	Content (%)
SiO_2	65.00-70.00
Al_2O_3	17.70
TiO_2	0.50
Fe_2O_3	1.60
FeO	1.20
Mn	0.05
MgO	0.65
CaO	2.80
Na_2O	4.30
K_2O	2.20
P_2O_5	0.22

Table 3.2 Surface area and pore volume of the Ca-modified and natural WRS

	BET surface area (m^2/g)	Langmuir surface area (m^2/g)	T-plot surface area (m^2/g)	Pore volume (ml/g)
Ca-modified WRS	4.56	2.85	8.49	0.021
Natural WRS	0.97	0.75	1.43	0.0034

Table 3.3 Isotherm constants and regression data of Langmuir and Freundlich isotherm models for ammonium adsorption on natural and Ca-modified WRS (adsorbent dose = 20 g/l, pH = 7.0; T = 35 ℃)

	Natural WRS	Ca-modified WRS
Freundlich		
K_f	0.0014	0.1304
n_f	0.9965	0.6134
R^2	0.9841	0.9762
Langmuir		
Q_m	23.98	45.45
b	0.0064	0.0026
R^2	0.9830	0.9646

Table 3.4 Kinetic parameters of Lagergren first-order and Ho's pseudo-second-order models for ammonium adsorption on natural and Ca-modified WRS (adsorbent dose = 20 g/l; C_0 = 5000 mg N/l; T = 35 ℃)

	Natural WRS	Ca-modified WRS
Lagergren first-order model		
q_e	9.954	5.960
k_1	0.0002	0.0044
R^2	0.6882	0.4149
Ho's pseudo-second-order model		
q_e	8.350	35.84
k_2	0.1510	0.0263
R^2	0.9999	0.9999

Chapter 4 Application of the modified porphyritic andesite in anaerobic digestion

4.1 Introduction

From the chapter 3, a modified porphyritic andesite with improved ammonium adsorption capacity was developed. In this we try to utilize the properties of the modified WRS to set up an anaerobic digestion system treating ammonium-rich swine waste with effective ammonium removal and stable bioactivity.

The objectives of this study include the following parts:

(1) Determine whether the modified porphyritic andesite could improve the anaerobic digestion efficiency comparing with the natural one.

(2) If the improved performance could be obtained by using the modified WRS, try to explain the reason.

(3) Investigate the potential utilization of the modified WRS in anaerobic digestion of ammonium-rich swine wastes.

4.2 Material and methods

4.2.1 Swine waste and seed sludge

Because a higher concentration of ammonium-rich swine waste was required, the raw swine waste used in the experiment was stale manure that had been kept at room temperature for over two years after it had been obtained from a pig farm located in Ibaraki province. Compared with fresh swine waste, stale manure has a higher concentration of ammonium; in fact, it can reach levels of up to 22310 mg/l.

The anaerobic digestion seed sludge was collected in a digested sludge tank from a sewage treatment plant in Ibaraki prefecture, Japan. The digested sludge was transferred into a plastic box and sealed, after which it was refrigerated under 4 ℃.

After the swine waste was diluted with tap water and its pH adjusted with 1 M HCl, it was inoculated with 20% sludge (w/w). The characteristics of the seed sludge and the diluted swine waste used for methane fermentation are shown in Table 4.1.

4.2.2 Natural and modified WRS

Natural WRS were obtained from Henan province, China. In order to improve the ammonium exchange capacity onto the natural WRS, salinization-calcination methods were used to modify the natural WRS. Firstly, the amount of 7g of WRS powder, 1g of bentonite, 1g of starch, and 1g of $CaCl_2$ were mixed to homogeneity. Deionized water was then added into the mixture to make a paste and the granulation procedure was carried out manually. After that, the obtained granules were dried at 105 ℃ for 24 h and calcined at 600 ℃ for 2 h. Finally, the modified WRS were cooled to room temperature for further studies.

4.2.3 Cation dissociation experiment

Natural and modified WRS were immersed in deionised water and ammonium solution at 35 ℃ for 3 days, with WRS-dosage of 20 g/l and ammonium concentration of 3550 mg N/l. Metal elements (K, Ca, Na, Mg, Fe, Ni, Co, Mn, Zn and Cu) dissociated from the natural and modified WRS or exchanged with ammonium were detected.

4.2.4 Anaerobic digestion experiment

A 300 ml Schott Duran bottle was used as bioreactor for the anaerobic digestion experiment. Methane fermentation was performed in four bioreactors with (1) natural WRS; (2) modified WRS; (3) calcium chloride; and (4) no additives. In the bioreactor with natural and modified WRS, 20 g/l WRS were fixed in a porous bag and suspend in the diluted swine waste. The configuration of the bioreactor referred to our previous study [51]. In the bioreactor with calcium chloride, 503.5 mg/l $CaCl_2$-chemicals were added into the digestion mixture. The experiments were performed in batch mode at 35 ℃ for 44 days. Each bioreactor contained 200 ml of diluted swine waste including 20% (w/w) digested sludge. Each group of experiments was performed in triplicate.

4.2.5 Analytical methods

The biogas was collected using three 50 ml plastic syringes, and the volume was read directly using the scale on the syringe. The gas composition was detected via gas chromatography (GC-8A, SHIMAZU, Japan) using a machine equipped with a thermal conductivity detector and a Porpapak Q column. Ammonium concentration was determined using an ion meter (Ti 9001, Toyo Chemical Laboratories Co. Ltd.). ATP value was detected using a Bac Titer-Glo^{TM} Microbial Cell Viability Assay (Promega, USA). COD, TS, VS, and TN were detected with standard methods [111], and pH was determined using a pH meter (SG8-ELK, SevenGo pro). Cations were measured by an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima-7300V, Perkin Elmer).The morphological images of the immobilized microorganism on the surface of the natural and modified WRS were acquired by scanning electron microscope (SEM) (JSM-6700F, JEOL, Japan).

4.3 Results and discussion

4.3.1 Cation dissociation of natural and modified WRS

Many cations can be dissociated from WRS, including Na, K, Ca and over 20 trace elements, which contribute the material with bioactivity [112]. These elements are necessary for the anaerobic digestion process because they are needed by microorganism to grow. Takashima and Speece [113] summarized the requirements of metal elements for methane fermentation, for example, Na and K support energy conservation and nutrient transport, Ca, Mg, Fe, Ni, Co, Cu, Mn and Zn are necessary for activity of dehydrogenase or methyltransferase or ATPase.

Table 4.2 shows cation dissociation capacity and ammonium exchange capacity of the natural and modified WRS. The results show that Na, K, Ca, Mg, Fe, Ni, Co, Cu, Mn and Zn dissociated from the natural and modified WRS after the material had been immersed in deionised water. WRS displayed the bioactivity considering the important roles of these elements for the growth of anaerobic microorganisms.

When the material was immersed in ammonium solution, certain cations were exchanged with ammonium. The concentration of these cations in ammonium solution was higher than that in deionized water. For the natural WRS, the concentration of K and Mg is two-fold higher and Ca increases by almost 15 times. For the modified WRS, the concentration of Na, K and Mg was over doubled and Ca^{2+} increased from 2.94 to 503.5 mg/l. It means that the cation dissociation from both natural and modified WRS was enhanced by ion exchange with ammonium. Besides that, as the addition of $CaCl_2$ in the modification process, the exchangeable Ca^{2+}on modified WRS is 14 times higher than that on natural WRS. 503.5 mg/l of Ca^{2+} was detected in the ammonium solution which means the main exchangeable cation is Ca^{2+} from the remarkable increments of Ca^{2+} comparing with other cations. Therefore, the modified WRS exhibited better cation dissociation performance than the nature WRS, especially when the material was immersed in ammonium solution. It was further proved by the concentration of ammonium decreasing from 3550 mg/l to 2904 mg/l in the modified WRS immersed solution, but from 3550 mg/l to 3389 mg/l in the natural WRS immersed one.

It was reported that Na played an important role in methanogenesis from methylamine, potassium acted as potent thermostabilizer and Ca^{2+} was required for stability of methyltransferase [114-116]. Schmidt and Ahring [117] found that methanosarcina were scarce in the absence of Mg^{2+} but increased with increasing Mg^{2+} up to 30 mM in a upflow anaerobic sludge blanket reactor. Therefore, the modified WRS is a promising bed material with bioactivity by dissociation of functional cations for the anaerobic digestion of ammonium-rich swine waste.

4.3.2 The performance of anaerobic digestion

To investigate the application of modified WRS in anaerobic digestion of ammonium-rich swine waste, methane fermentation was carried out in a bioreactor with modified WRS at an initial NH_4^+-N concentration of 3550 mg/l, temperature of 35 ℃.The performance of methane yield was compared with the bioreactors containing natural WRS and no additives (control) at the same operating conditions. From section 4.3.1, there is a high concentration of Ca^{2+} (503.5 mg/l) dissociated into the liquid phase. To elucidate the effect of Ca^{2+}, methane fermentation was also performed in a bioreactor with the addition of $CaCl_2$ at the same operating conditions.

Fig.4.1 shows the biogas production and corresponding methane concentration in the four bioreactors. It can be seen that the start-up period is 7 days in the bioreactor with modified WRS. Beginning on the 8th day, biogas production increase gradually to the daily maximum of 1325 ml/l on the 16th day, and the corresponding methane concentration increased from 6.73% to 82.32%. Thereafter, biogas production decrease day by day, but the methane concentration remains stable at around 80% until the end of the batch experiment. However, the start-up time is 16, 18 and 22 days in the bioreactors with Ca^{2+}, natural WRS and no additives, and the maximum daily methane yield is 600 ml/l, 850 ml/l, and 575 ml/l, respectively. In addition, the variation tendencies of methane concentration are in line with their biogas production and do not sustain a stable value during the 44 days of the experiment. It can be concluded the bioreactor with modified WRS exhibited better performance than the three others in terms of shortest start-up time, highest biogas yield and stable methane concentration.

In addition, the performance of the bioreactor with Ca^{2+} is better than control by the advanced start-up time and enhanced daily biogas production (Fig. 4.3). This means that Ca^{2+} has a positive effect on the anaerobic digestion of ammonium-rich swine waste. This result is supported by Ahn [101] who found that the performance of anaerobic digestion improved with calcium addition, and methane production enhanced by increasing the concentration of calcium from 0 to 3 g/l. It is attributed to the functional role of calcium in stabilizing enzyme activity of anaerobic microorganisms or mitigating ammonium toxicity as an antagonistic ion [26, 118].Therefore, Ca^{2+}dissociation from the modified WRS is one of the factors promoting the anaerobic digestion efficiency of ammonium-rich swine waste.

Fig. 4.2 shows the variation of ammonium in the four reactors during 44 days of the experiment. Ammonium concentration shows an increasing tendency in all bioreactors, and the value is from initial 3550 mg/l to the end over 3700 mg/l, because ammonium is produced during the anaerobic digestion process by the biological degradation of the nitrogenous matter [119]. However, 17.2% ammonium is reduced in the fermentation liquid, and the concentration is 2960 mg N/l on day 3 in the bioreactor with modified WRS. It demonstrated that ammonium which inhibits the microorganisms was partially removed by modified WRS. The similar phenomenon appears in the bioreactor with natural WRS, however, only 150 mg/l NH_4^+-N is reduced, and the concentration is 3400 mg N/l on day 3. These results suggested the modified WRS is a good adsorbent for ammonium removal in the anaerobic digestion of ammonium-rich swine waste. Effective reducing of ammonium will contribute to shortening start-up time and improving anaerobic digestion performance [10, 120]. In this study, the effective ammonium removal by the modified WRS is another factor enhancing the anaerobic digestion performance of ammonium-rich swine waste.

4.3.3 Microorganism activity

An acceptable anaerobic digestion system depends on the adequate amount and high metabolic activity of methanogens for high-efficiency conversion of organic carbon to methane. ATP is an important parameter to reflect the performance of anaerobic digestion, as an indicator of metabolically active cells and an index of microbial density [89]. The larger quantities of microbes would contribute to the higher concentration of ATP.

In this study, ATP was tested on the surface of the natural and modified WRS, and in the liquid phase of the four bioreactors at the end of the digestion experiment (Fig. 4.3). It shows that ATP concentration in the liquid phase of the bioreactors with Ca^{2+} and no additives is slightly higher than that in the bioreactors with natural and modified WRS. However, the value on the surface of the modified and natural WRS is much higher. It can be inferred that most of microbes growing on the surface of the support materials. Moreover, ATP level on the surface of the modified WRS is higher than that on the surface of the natural one. This result is further confirmed by the SEM photographs of microbes on the natural and modified WRS (Fig. 4.4). It is clear that more microbes immobilized on the surface of the modified WRS, and the diversity of morphologies can be found. This microphotograph indicates that the immobilized microorganisms primarily composed of rods of Clostridium sp., coccus, rods of Bacillus sp., and coccobacillus of Ruminococcus sp.-like bacteria [91, 121]. The diversity of methanogens could play an important role in methane fermentation. Kuo and Shu [122] reported that a system with immobilized cells could tolerate higher levels of toxic material compared to a suspended growth system. Therefore, the bioreactor with the modified WRS has a high tolerance to ammonium. The big-amount and high-activity microorganisms on the surface of the modified WRS contributed to improving the anaerobic digestion performance of ammonium-rich swine waste.

4.3.4 Effectiveness of anaerobic digestion of ammonium-rich swine waste in the bioreactor with modified WRS

Fig. 4.5 shows total methane yield in terms of VS and COD removal efficiency for all batch experiments. In this study, the methane yield and COD removal figures are 359.71 ml/g-VS and 67.99%, respectively, for the bioreactor with modified WRS. At the relatively high initial concentrations of ammonium and COD, methane yield was higher than potential methane yield of 300 $\pm$20 ml/g-VS reported by Hansen et al. [73], and maximum methane yield of 244-343 ml/g-VS from swine manure reported by Vedrenne et al. [123].

Zhang and Jahng [124] reported that anaerobic digestion of piggery wastewater could be improved by ammonia stripping using CaO. Their study yielded COD removal efficiency of 64.0$\pm$0.3% with initial COD and ammonium concentration of 80,400 m g/l and 2520 mg/l respectively. Here, however, 67.99% of COD was removed at the higher initial COD and ammonium concentration of 80,537 mg/l and 3550 mg/l. In addition, methane yield and COD removal efficiency achieved with modified WRS is 58% and 46% higher than that achieved with no additives under the same running conditions.

Besides, compared with our previous study [51], bioreactor with modified WRS demonstrated better performance than bioreactor with zeolite A-3 in terms of faster start-up time, higher COD removal efficiency, and higher accumulated methane yield (Table 4.3). This could ascribe to the effective functional ions dissociation from the modified WRS. Compared with the modified WRS, zeolite has no bioactivity by ions dissociation. Besides that, the cost of the modified WRS was much cheaper than zeolite A-3. Based on the power consumption and utilized chemicals during the processes of modification, the rough estimate of the modified WRS was 3.0 yen/g, while the zeolite A-3 was 7.0 yen/g. The calculation was based on the small scale test. Furthermore, the modification cost could be much reduced in the commercial production.

Therefore, modified WRS is an effective ammonium adsorbent and bed material in the anaerobic digestion of ammonium-rich swine waste. The good performance is attributed to (1) partially ammonium uptake by the modified WRS which reduced the inhibition of ammonium; (2) cation dissociation, especially Ca^{2+} improved the activity of anaerobic microorganisms; and (3) a big amount of microbes immobilized on the surface of the material and the diversity of methanogens played an important role in enhancing the tolerance of the system to high levels of ammonium.

4.4 Summary

In this study a modified WRS was applied to the efficient anaerobic digestion of ammonium-rich swine waste. Cation dissociation improved the bioactivity of microbes. A big amount of microorganisms growing on the surface of the modified WRS and the diversity of methanogens enhanced the toxic tolerance of the anaerobic system. Based on its excellent performance in the anaerobic digestion system, modified WRS could be a feasible bed material in improving the anaerobic digestion performance of ammonium-rich swine waste.

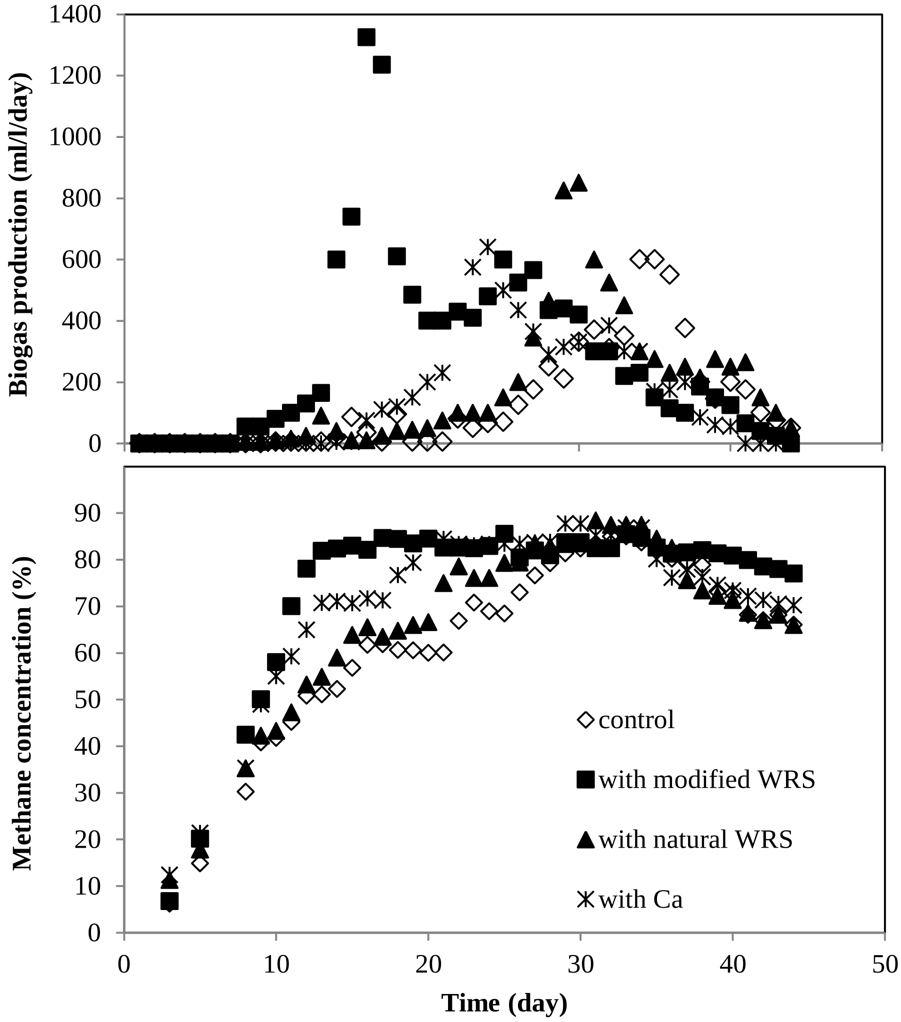


Figure 4.1 Biogas production and methane concentration for the anaerobic digestion of ammonium-rich swine waste at 35 ℃

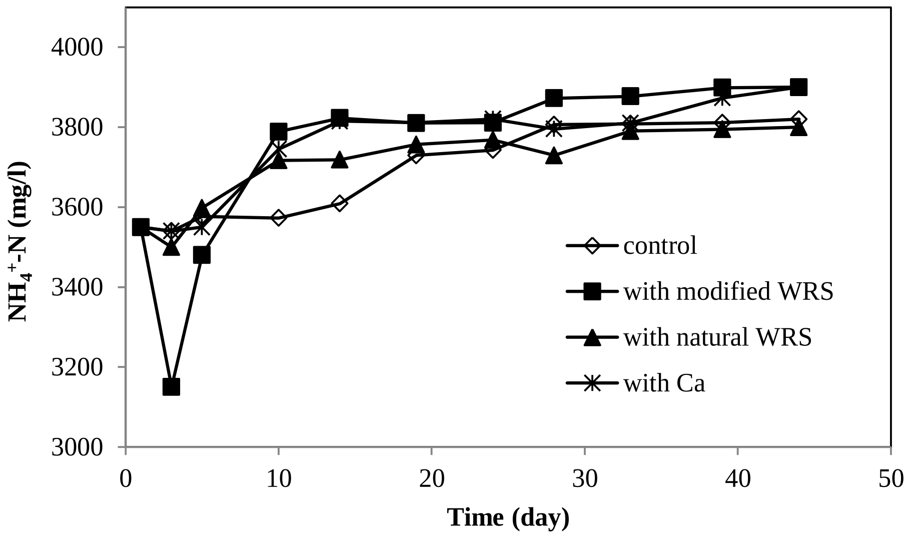


Figure 4.2 Ammonium concentration for the four bioreactors during the operational period.

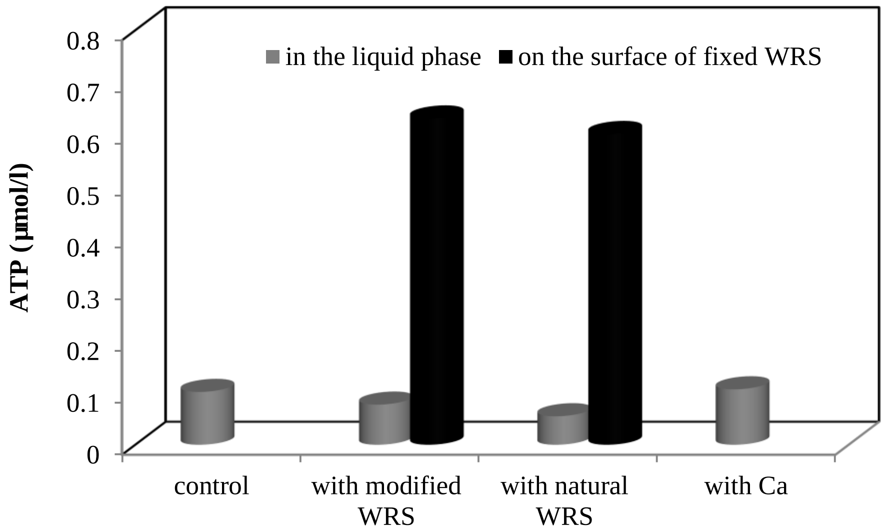


Figure 4.3 ATP values for the four bioreactors at the end of the experiment.

(a)

(b)

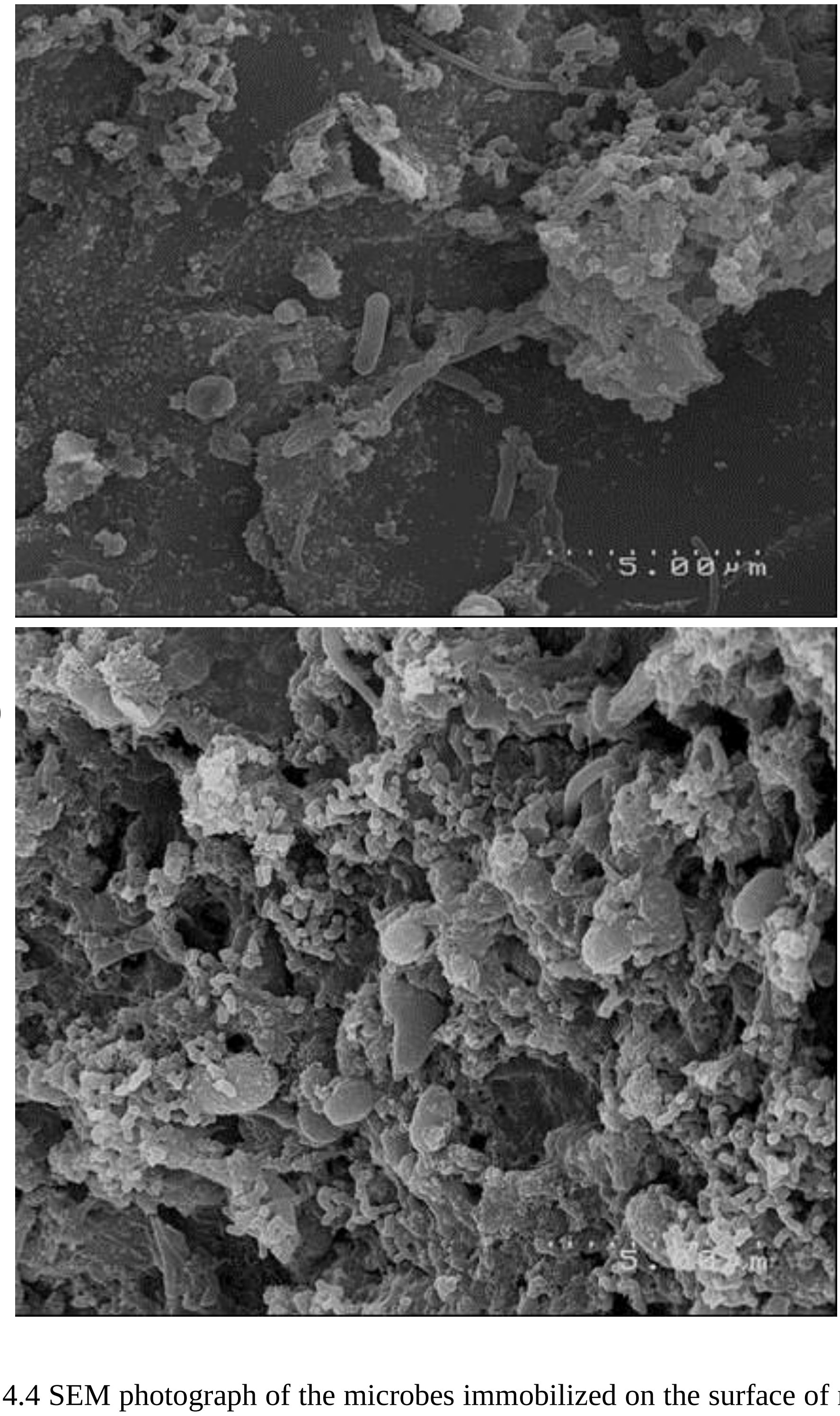


Figure 4.4 SEM photograph of the microbes immobilized on the surface of natural (a) and modified WRS (b). Scale bar is 5 μm; magnification, 4000×.

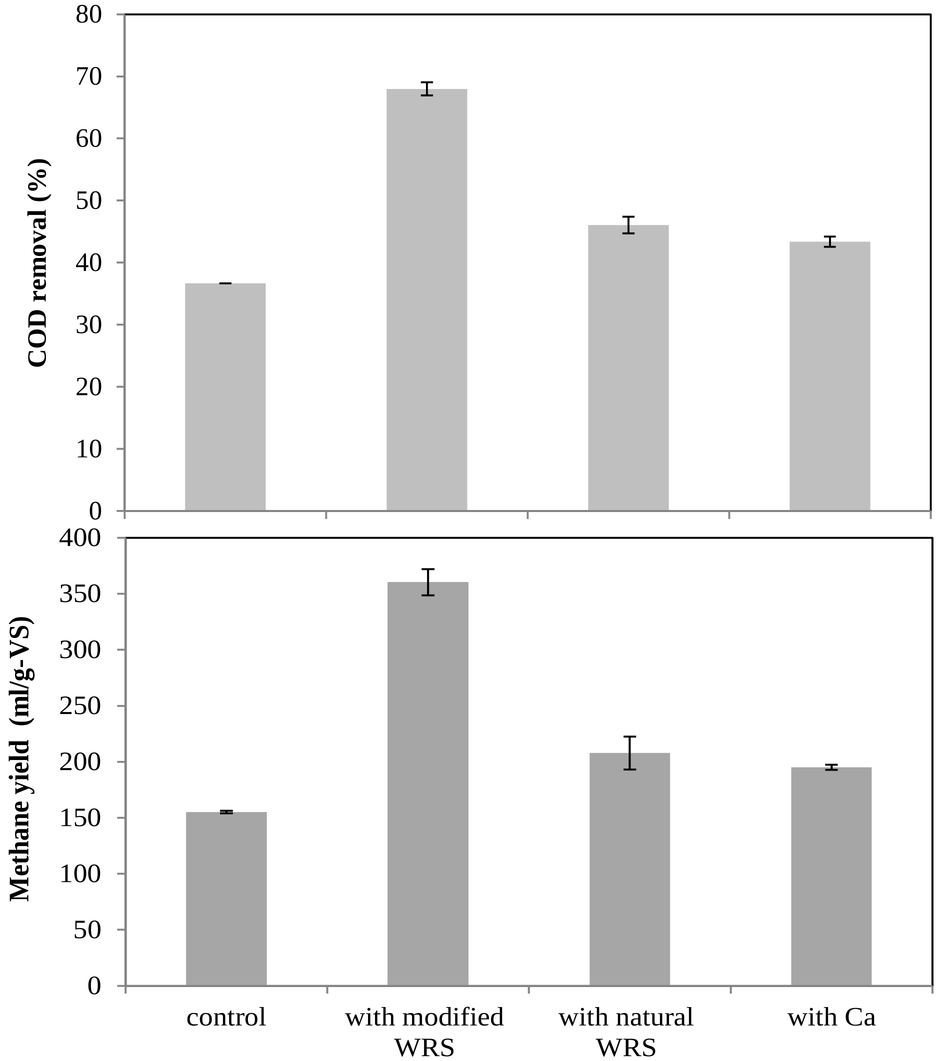


Figure 4.5 COD removal and methane yield in the four bioreactors. The bars designate standard deviations (95% confidence, t-test).

Table 4.1 The characteristics of seed sludge and swine waste used in the experiments after dilution with deionized water and with adjusted pH

Parameters	Digestion sludge	Swine wastes
COD (mg/l)	6500	80537
TN (mg/l)	5489	9442
TS (mg/l)	13292	37920
VS (mg/l)	9500	29000
NH_4^+-N (mg/l)	1547	3550
pH	7.11	7.05

Table 4.2 The concentration of metal elements from natural and modified WRS

Element	Natural WRS [a] (mg/l)	Modified WRS [a] (mg/l)	Natural WRS [b] (mg/l)	Modified WRS [b] (mg/l)
Na	3.379	2.971	3.568	7.980
K	1.245	1.268	2.481	2.610
Mg	0.701	0.731	1.651	2.469
Ca	2.471	2.940	35.88	503.5
Mn	0.382	0.401	0.323	0.408
Fe	0.166	0.105	0.181	0.115
Co	<0.01	<0.01	<0.01	<0.01
Ni	<0.01	<0.01	<0.01	<0.01
Cu	0.163	0.237	0.197	0.237
Zn	0.034	0.032	0.034	0.039

[a] WRS have been immersed in the deionized water for 3 days, at 35 ℃

[b] WRS have been immersed in 3550 mg N/l of ammonium solution for 3 days, at 35 ℃

Table 4.3 Comparing the performance of anaerobic digestion with modified WRS and zeolite A-3

	Start-up time (day)	Accumulated methane yield (ml/g-VS)	COD removal efficiency (%)	Material cost (yen/g)	Reference
Modified WRS	7	360.3	68.0	3.0	This study
Zeolite A-3	14	178.5	46.6	7.0	Wang et al. (2011)

Chapter 5 Conclusions

In this study, a modified porphyritic andesite fixed bioreactor was developed for the anaerobic digestion of ammonium-rich swine waste. Several advantages make the bioreactor with good performance in methane production and COD removal and application prospects, such as (a) effective ammonium removal by the modified porphyritic andesite, (b) functional cation dissociation from the modified porphyritic andesite improve the bioactivity of the microorganism, (c) sufficient mechanical strength of the modified material to retain its physical integrity under long-time anaerobic conditions, (d) the inexpensive preparation process for the modification process from clay material, (e) the configuration of the bioreactor make the ammonium-saturated adsorbent easy be replaced.

5.1 The performance of the fixed zeolite bioreactor for anaerobic digestion of ammonium-rich swine wastes

As zeolite is a typical and general ammonium adsorbent, a fixed zeolite bioreactor was developed for anaerobic digestion of ammonium-rich swine wastes. From the batch study of anaerobic digestion in three bioreactors: fixed zeolite bioreactor, sunken zeolite bioreactor and without zeolite bioreactor, the following conclusions were obtained

(1) The fixed zeolite bioreactor exhibited good performance, with startup time on

the 14th day and methane production of 178.5 ml/g-VS during all 32 days of the experiment at 35 ℃.

(2) This bioreactor significantly shortened startup time, enhanced methane gas yield more than twofold and made COD removal more efficient than under the other models.

(3) This bioreactor reduced the inhibition of high ammonium concentration during the anaerobic digestion of ammonium-rich swine wastes via effective ammonium removal and the immobilization of microorganisms.

5.2 The development of modified porphyritic andesite

As the zeolite A-3 is a kind of expensive synthetic product from Woko Company, a substitute material with high ammonium adsorption capacity and bioactivity as well as low cost need to be developed. On the basis of the above purpose, a modified porphyritic andesite was developed and its properties were investigated. The conclusions were draw as follows:

(1) A integrated Ca-salt treatment and calcination method (with 10% $CaCl_2$ and calcined at 600 ℃ for 2 h) was used for the modification of porphyritic andesite

(2) Compared with the natural WRS, the surface of the Ca-modified WRS is rougher and pores diameters are larger, which indicates that a porous texture with irregularly defined channels was formed.

(3) The specific surface area of the Ca-modified WRS was determined to be 4.56 sq. m/g, and the maximum NH_4^+-N adsorption capacity was determined to be 45.45

mg/g.

(4) The kinetic data indicated that ammonium adsorption by Ca-modified WRS obeys pseudo-second-order kinetics, which suggests a chemisorption process in this experiment.

(5) The ammonium adsorption capacity remained constant at a pH range from 5.0 to 9.0, which indicates that Ca-modified WRS is a promising material for various applications.

5.3 The application of the modified porphyritic andesite in anaerobic digestion of ammonium-rich swine wastes

As the advantages of the modified porphyritic andesite such as high ammonium adsorption capacity, big surface area, and pH-stability, the modified porphyritic andesite was used as ammonium adsorbent and bed material in anaerobic digestion of ammonium-rich swine wastes. The performance of these bioreactors with modified WRS, natural WRS, calcium chloride and no additives were investigated, and the conclusions were draw as follows:

(1) The modified WRS fixed bioreactor exhibited the best performance, with start-up time on the 7th day, methane yield of 359.71 ml/g-VS, and COD removal of 67.99% during all 44 days of the experiment at 35 ℃.

(2) The effective ammonium adsorption and essential ions dissociation for microorganisms by modified WRS, as well as the immobilization of microbial on the surface of the modified WRS played a great role on the high efficiency anaerobic digestion of ammonium-rich swine waste.

(3) Based on the power consumption and utilized chemicals during the processes of modification, the rough estimate of the modified WRS was 3.0 yen/g, which was much cheaper than zeolite A-3.

Therefore, on the basis of its excellent performance in high methane production, high COD removal, low cost and easy operation, the modified WRS fixed bioreactor could be recommend for further use in improving the anaerobic digestion of ammonium-rich swine waste.

5.4 Further research

This research developed a novel bioreactor for anaerobic digestion of ammonium-rich swine wastes and its performance was investigated at batch mode. Form the consideration of practical application, continuous experiment at pilot-scale is recommended for further research. The following directions are concluded:

(1) A pilot-scale fixed modified-WRS bioreactor will be set up for the continuous anaerobic digestion of ammonium-rich swine waste. Different OLR and HRT will be tested in continuous experiment to investigate the optimum operating conditions. A suitable replacement cycle of the adsorbent will be determined for sustainable efficient anaerobic digestion of ammonium-rich swine waste.

(2) The ammonium saturated adsorbent whether can be used as an effective fertilizer in agriculture will be investigated.

References

[1] M. Yao, Profile of China pig industry and development, in: Global Pig industry fourm-2009 developement of pig industry in China, 2009.

[2] P. R.G, Fungal population levels in soils of commercial swine waste disposal sites and relationships to soil nutrient concentrations, Applied Soil Ecology, 38 (2008) 223-229.

[3] M. S.J, Treatment of swine wastes by a high-rate-modified-anaerobic-process (HRAMP), Bioresource Technology, 53 (1995) 207-210.

[4] R. Fleming, D. Hocking, M. MacAlpine, J. Johnston, Investigation Of Manure Production In Typical 3-Site Hog Facilities, in, 1999.

[5] X. Wang, Diffuse pollution from livestock production in China, Chinese Journal of Geochemistry, 24 (2005) 189-193.

[6] P.K.S. Shin, N.W.Y. Lam, R.S.S. Wu, P.Y. Qian, S.G. Cheung, Spatio-temporal changes of marine macrobenthic community in sub-tropical waters upon recovery from eutrophication. I. Sediment quality and community structure, Marine Pollution Bulletin, 56 (2008) 282-296.

[7] H.-D. Ryu, S.-I. Lee, Application of struvite precipitation as a pretreatment in treating swine wastewater, Process Biochemistry, 45 (2010) 563-572.

[8] R. Khaleel, K.R. Reddy, M.R. Overcash, Transport of potential pollutants in runoff water from land areas receiving animal wastes: A review, Water Research, 14 (1980) 421-436.

[9] A.J. Ward, P.J. Hobbs, P.J. Holliman, D.L. Jones, Optimisation of the anaerobic digestion of agricultural resources, Bioresource Technology, 99 (2008) 7928-7940.

[10] C. Resch, A. Wörl, R. Waltenberger, R. Braun, R. Kirchmayr, Enhancement options for the utilisation of nitrogen rich animal by-products in anaerobic digestion, Bioresource Technology, 102 (2011) 2503-2510.

[11] S.G. Pavlostathis, E. Giraldo-Gomez, Kinetics of anaerobic treatment: A critical review, Critical Reviews in Environmental Control, 21 (1991) 411-490.

[12] W. Gujer, A.J.B. Zehnder, Conversion processes in anaerobic digestion, Water Science and Technol. 15 (1983) 127-167, (1983).

[13] H. Gavala, I. Angelidaki, B. Ahring, Kinetics and Modeling of Anaerobic Digestion Process Biomethanation I, in: B. Ahring, I. Angelidaki, E. de Macario, H. Gavala, J. Hofman-Bang, A. Macario, S. Elferink, L. Raskin, A. Stams, P. Westermann, D. Zheng (Eds.), Springer Berlin / Heidelberg, 2003, pp. 57-93.

[14] G.E. Symons, A.M. Buswell, The Methane Fermentation of Carbohydrates1,2, Journal of the American Chemical Society, 55 (1933) 2028-2036.

[15] M. Kayhanian, Ammonia Inhibition in High-Solids Biogasification: An Overview and Practical Solutions, Environmental Technology, 20 (1999) 355-365.

[16] C. Wittmann, A.P. Zeng, W.D. Deckwer, Growth inhibition by ammonia and use of a pH-controlled feeding strategy for the effective cultivation of Mycobacterium chlorophenolicum, Applied Microbiology and Biotechnology, 44 (1995) 519-525.

[17] W.M. Wiegant, G. Zeeman, The mechanism of ammonia inhibition in the thermophilic digestion of livestock wastes, Agricultural Wastes, 16 (1986) 243-253.

[18] I.W. Koster, G. Lettinga, The influence of ammonium-nitrogen on the specific activity of palletized

methanogenic sludge, Agriculture Wastes, 9 (1984) 205-216.
[19] M. Kayhanian, Performance of a high-solids anaerobic digestion process under various ammonia concentrations, Journal of Chemical Technology & Biotechnology, 59 (1994) 349-352.
[20] I.W. Koster, G. Lettinga, Anaerobic digestion at extreme ammonia concentrations, Biological Wastes, 25 (1988) 51-59.
[21] R.E. Speece, Anaerobic biotechnology for industrial wastewater treatment, Environmental Science & Technology, 17 (1983) 416A-427A.
[22] I. Angelidaki, B.K. Ahring, Thermophilic anaerobic digestion of livestock waste: the effect of ammonia, Applied Microbiology and Biotechnology, 38 (1993) 560-564.
[23] R. Borja, E. Sánchez, M.M. Durán, Effect of the clay mineral zeolite on ammonia inhibition of anaerobic thermophilic reactors treating cattle manure, Journal of Environmental Science and Health . Part A: Environmental Science and Engineering and Toxicology, 31 (1996) 479-500.
[24] K.F. Jarrell, M. Saulnier, A. Ley, Inhibition of methanogenesis in pure cultures by ammonia, fatty acids, and heavy metals, and protection against heavy metal toxicity by sewage sludge, Canadian Journal of Microbiology, 33 (1987) 551-554.
[25] T. Liu, S. Sung, Ammonia inhibition on thermophilic aceticlastic methanogens, Water Science and Technology, 45 (2002) 113-120.
[26] P.L. Mccarty, R.E. Mckinney, Salt toxicity in anaerobic digestion, Water Pollution Control Federation, 33 (1961) 399-415.
[27] G. Zeeman, W. Wiegant, M. Koster-Treffers, G. Lettinga, The influence of the total ammonia concentration on thermophilic digestion of cow manure., Agriculture research, 14 (1985) 19-35.
[28] A.G. Hashimoto, Ammonia inhibition of methanogenesis from cattle wastes, Agricultural Wastes, 17 (1986) 241-261.
[29] P.N. Hobson, B.G. Shaw, Inhibition of methane production by Methanobacterium formicicum, Water Research, 10 (1976) 849-852.
[30] A. Van Velsen, Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations., Water Research, 13 (1979) 995-999.
[31] R. Borja, E. Sánchez, P. Weiland, Influence of ammonia concentration on thermophilic anaerobic digestion of cattle manure in upflow anaerobic sludge blanket (UASB) reactors, Process Biochemistry, 31 (1996) 477-483.
[32] B. Calli, B. Mertoglu, B. Inanc, O. Yenigun, Effects of high free ammonia concentrations on the performances of anaerobic bioreactors, Process Biochemistry, 40 (2005) 1285-1292.
[33] S.K. Bhattacharya, The effect of ammonia on methane fermentation processes, 1989.
[34] R. Braun, P. Huber, J. Meyrath, Ammonia toxicity in liquid piggery manure digestion, Biotechnology Letters, 3 (1981) 159-164.
[35] M.A. de la Rubia, L.I. Romero, D. Sales, M. Perez, Pilot-scale anaerobic thermophilic digester treating municipal sludge, AICHE Journal, 52 (2006) 402-407.
[36] G.F. Parkin, S.W. Miller, Response of methane fermentation to continuous addition of selected industrial toxicants., in: Proceedings of the 37th Purdue Industrial Waste Conference, West Lafayette, 1983, pp. 729-744.
[37] A.G. Hashimoto, Methane from swine manure: Effect of temperature and influent substrate concentration on kinetic parameter (K), Agricultural Wastes, 9 (1984) 299-308.
[38] E. Sánchez, R. Borja, P. Weiland, L. Travieso, A. Martín, Effect of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal in the batch anaerobic

digestion process of cattle manure, Bioprocess and Biosystems Engineering, 22 (2000) 247-252.
[39] I. Angelidaki, B.K. Ahring, Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature, Water Research, 28 (1994) 727-731.
[40] K.H. Hansen, I. Angelidaki, B.K. Ahring, Improving thermophilic anaerobic digestion of swine manure, Water Research, 33 (1999) 1805-1810.
[41] N.R. Melbinger, J. Donnellon, Toxic effects of ammonia-nitrogen in high rate digestion. , Water Pollution Control Federation, 43 (1971) 1658-1670.
[42] E.J. Kroeker, D.D. Schulte, A.B. Sparling, H.M. Lapp, Anaerobic treatment process stability, Water Pollution Control Fed, 51 (1979) 718-727.
[43] L.A. de Baere, M. Devocht, P. Van Assche, W. Verstraete, Influence of high NaCl and NH4Cl salt levels on methanogenic associations, Water Research, 18 (1984) 543-548.
[44] H.M. Janus, H.F. van der Roest, Don't reject the idea of treatingreject water, Water Science and Technology, 35 (1997) 27-34.
[45] O. Kabdasli, Ï.Ö. Tünay, S. Yilmaz, O. Arikan, Ammonia removal from young landfill leachate by magnesium ammonium phosphate precipitation and air stripping, Water Science and Technology, 41 (2000) 237-240.
[46] K. Cheung, L. Chu, M. Wong, Ammonia stripping as a pretreatment for landfill leachate, Water, Air, & Soil Pollution, 94 (1997) 209-221.
[47] H. Schiweck, C. Nähle, Removal of ammonia from condensates and surplus condenser water by stripping with air, Zuckerindustrie, 115 (1990) 639-647.
[48] P.H. Liao, A. Chen, K.V. Lo, Removal of nitrogen from swine manure wastewaters by ammonia stripping, Bioresource Technology, 54 (1995) 17-20.
[49] A. Rinzema, J. van Lier, G. Lettinga, Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor, Enzyme and Microbial Technology, 10 (1988) 24-32.
[50] I. Angelidaki, B.K. Ahring, Effect of the clay mineral bentonite on ammonia inhibition of anaerobic thermophilic reactors degrading animal waste, Biodegradation, 3 (1993) 409-414.
[51] Q. Wang, Y. Yang, C. Yu, H. Huang, M. Kim, C. Feng, Z. Zhang, Study on a fixed zeolite bioreactor for anaerobic digestion of ammonium-rich swine wastes, Bioresource Technology, 102 (2011) 7064-7068.
[52] D. Fu, Natural zeolite and its future use in environmental proctection, China Non-Metallic Mining Industry Herald 28 (2002) 30-32.
[53] C. Tada, Y. Yang, T. Hanaoka, A. Sonoda, K. Ooi, S. Sawayama, Effect of natural zeolite on methane production for anaerobic digestion of ammonium rich organic sludge, Bioresource Technology, 96 (2005) 459-464.
[54] S. Montalvo, L. Guerrero, R. Borja, L. Travieso, E. Sánchez, F. Díaz, Use of natural zeolite at different doses and dosage procedures in batch and continuous anaerobic digestion of synthetic and swine wastes, Resources, Conservation and Recycling, 47 (2006) 26-41.
[55] Z. Milán, E. Sánchez, P. Weiland, R. Borja, A. Martín, K. Ilangovan, Influence of different natural zeolite concentrations on the anaerobic digestion of piggery waste, Bioresource Technology, 80 (2001) 37-43.
[56] T.A. Kotsopoulos, X. Karamanlis, D. Dotas, G.G. Martzopoulos, The impact of different natural zeolite concentrations on the methane production in thermophilic anaerobic digestion of pig waste, Biosystems Engineering, 99 (2008) 105-111.
[57] N.O. Nelson, R.L. Mikkelsen, D.L. Hesterberg, Struvite precipitation in anaerobic swine lagoon

liquid: effect of pH and Mg:P ratio and determination of rate constant, Bioresource Technology, 89 (2003) 229-236.
[58] S.I. Lee, S.Y. Weon, C.W. Lee, B. Koopman, Removal of nitrogen and phosphate from wastewater by addition of bittern, Chemosphere, 51 (2003) 265-271.
[59] C. Rico, H. García, J.L. Rico, Physical–anaerobic–chemical process for treatment of dairy cattle manure, Bioresource Technology, 102 (2011) 2143-2150.
[60] N. Marti, A. Bouzas, A. Seco, J. Ferrer, Struvite precipitation assessment in anaerobic digestion processes, Chemical Engineering Journal, 141 (2008) 67-74.
[61] J.J. Lee, C.U. Choi, M.J. Lee, I.H. Chung, D.S. Kim, A study of NH3-N and P refixation by struvite formation in hybrid anaerobic reactor., Water Science and Technology, 49 (2004) 207-214.
[62] N.I. Krylova, R.E. Khabiboulline, R.P. Naumova, M.A. Nagel, The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure, Journal of Chemical Technology & Biotechnology, 70 (1997) 99-105.
[63] M.S.M. Jetten, M. Strous, K.T. van de Pas-Schoonen, J. Schalk, U.G.J.M. van Dongen, A.A. van de Graaf, S. Logemann, G. Muyzer, M.C.M. van Loosdrecht, J.G. Kuenen, The anaerobic oxidation of ammonium, FEMS Microbiology Reviews, 22 (1998) 421-437.
[64] X. Dong, E.W. Tollner, Evaluation of Anammox and denitrification during anaerobic digestion of poultry manure, Bioresource Technology, 86 (2003) 139-145.
[65] M. Waki, T. Tokutomi, H. Yokoyama, Y. Tanaka, Nitrogen removal from animal waste treatment water by anammox enrichment, Bioresource Technology, 98 (2007) 2775-2780.
[66] D. Karakashev, J.E. Schmidt, I. Angelidaki, Innovative process scheme for removal of organic matter, phosphorus and nitrogen from pig manure, Water Research, 42 (2008) 4083-4090.
[67] Z. Yang, S. Zhou, Y. Sun, Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor, Journal of Hazardous Materials, 169 (2009) 113-118.
[68] S.W.H. Van Hulle, E.I.P. Volcke, J.L. Teruel, B. Donckels, M.C.M. van Loosdrecht, P.A. Vanrolleghem, Influence of temperature and pH on the kinetics of the Sharon nitritation process, Journal of Chemical Technology & Biotechnology, 82 (2007) 471-480.
[69] A. Dapena-Mora, I. Fernández, J.L. Campos, A. Mosquera-Corral, R. Méndez, M.S.M. Jetten, Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production, Enzyme and Microbial Technology, 40 (2007) 859-865.
[70] E. Sánchez, Z. Milán, R. Borja, P. Weiland, X. Rodriguez, Piggery waste treatment by anaerobic digestion and nutrient removal by ionic exchange, Resources, Conservation and Recycling, 15 (1995) 235-244.
[71] C.J. Banks, A.M. Salter, M. Chesshire, Potential of anaerobic digestion for mitigation of greenhouse gas emissions and production of renewable energy from agriculture: barriers and incentives to widespread adoption in Europe., Water Science and Technology, 55 (2007) 165-173.
[72] H.B. Nielsen, I. Angelidaki, Strategies for optimizing recovery of the biogas process following ammonia inhibition, Bioresource Technology, 99 (2008) 7995-8001.
[73] K.H. Hansen, I. Angelidaki, B.K. Ahring, Anaerobic digestion of swine manure: inhibition by ammonia, Water Research, 32 (1998) 5-12.
[74] A.F.M. Van Velsen, Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations, Water Research, 13 (1979) 995-999.
[75] I.W. Koster, G. Lettinga, The influence of ammonium-nitrogen on the specific activity of pelletized

methanogenic sludge, Agricultural Wastes, 9 (1984) 205-216.
[76] P.C. Kadam, D.R. Boone, Influence of pH on ammonia accumulation and toxicity in Halophilic, Methylotrophic Methanogens, Applied and Environmental Microbiology, 62 (1996) 4486-4492.
[77] J.J. Lay, Y.Y. Li, T. Noike, The influence of pH and ammonia concentration on the methane production in high-solids digestion processes, Water Environment Research, 70 (1998) 1075-1082.
[78] F. Abouelenien, W. Fujiwara, Y. Namba, M. Kosseva, N. Nishio, Y. Nakashimada, Improved methane fermentation of chicken manure via ammonia removal by biogas recycle, Bioresource Technology, 101 (2010) 6368-6373.
[79] A. Akram, D.C. Stuckey, Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC), Process Biochemistry, 43 (2008) 93-102.
[80] I. Angelidaki, S.P. Petersen, B.K. Ahring, Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite, Applied Microbiology and Biotechnology, 33 (1990) 469-472.
[81] S. Nikolaeva, E. Sánchez, R. Borja, L. Travieso, P. Weiland, Z. Milán, Treatment of piggery waste by anaerobic fixed bed reactor and zeolite bed filter in a tropical climate: a pilot scale study, Process Biochemistry, 38 (2002) 405-409.
[82] Y. Yang, C. Tada, M.S. Miah, K. Tsukahara, T. Yagishita, S. Sawayama, Influence of bed materials on methanogenic characteristics and immobilized microbes in anaerobic digester, Materials Science and Engineering: C, 24 (2004) 413-419.
[83] Q. Du, S. Liu, Z. Cao, Y. Wang, Ammonia removal from aqueous solution using natural Chinese clinoptilolite, Separation and Purification Technology, 44 (2005) 229-234.
[84] C.-H. Liu, K.V. Lo, Ammonia removal from composting leachate using zeolite.II. Characterization of the zeolite, Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 36 (2001) 1671-1688.
[85] E.L. Cooney, N.A. Booker, D.C. Shallcross, G.W. Stevens, Ammonia Removal from Wastewaters Using Natural Australian Zeolite. I. Characterization of the Zeolite, Separation Science and Technology, 34 (1999) 2307-2327.
[86] A.A. Halim, H.A. Aziz, M.A.M. Johari, K.S. Ariffin, Comparison study of ammonia and COD adsorption on zeolite, activated carbon and composite materials in landfill leachate treatment, Desalination, 262 (2010) 31-35.
[87] M. Kayhanian, Ammonia inhibition in high-solids biogasification: an overview and practical Environmental Technology 20 (1999) 355-365.
[88] C. Hong, ATP content and biomass activity in sequential anaerobic/aerobic reactors, Journal of Zhejiang University - Science A, 5 (2004) 727-732.
[89] C.P. Chu, D.J. Lee, B.-V. Chang, C.H. You, C.S. Liao, J.H. Tay, Anaerobic digestion of polyelectrolyte flocculated waste activated sludge, Chemosphere, 53 (2003) 757-764.
[90] Y.-C. Chung, J.B. Neethling, ATP as a Measure of Anaerobic Sludge Digester Activity, Water Pollution Control Federation, 60 (1988) 107-112.
[91] Y. Yang, K. Tsukahara, S. Sawayama, Biodegradation and methane production from glycerol-containing synthetic wastes with fixed-bed bioreactor under mesophilic and thermophilic anaerobic conditions, Process Biochemistry, 43 (2008) 362-367.
[92] H.B. Moller, S.G. Sommer, B.K. Ahring, Methane productivity of manure, straw and solid fractions of manure, Biomass and Bioenergy, 26 (2004) 485-495.
[93] I. Angelidaki, B.K. Ahring, Methods for increasing the biogas potential from the recalcitrant

organic matter contained in manure, Water Science and Technology 41 (2000) 189-194.
[94] N.-C. Kao, M.-K. Wang, P.-N. Chiang, S.-S. Chang, Characterization of wheat-rice-stone developed from porphyritic hornblende andesite, Applied Clay Science, 23 (2003) 337-346.
[95] W.-T. Tsai, T.-Y. Su, H.-C. Hsu, K.-Y. Lin, C.-M. Lin, T.-H. Tai, Preparation of mesoporous solids by acid treatment of a porphyritic andesite (wheat-rice-stone), Microporous and Mesoporous Materials, 102 (2007) 196-203.
[96] F. Guang-hua, China's Maifanshi resources and their development and research, Bulletin of Mineralogy, Petrology and Geochemistry, 20 (2001) 131-135.
[97] F. Cheng, M. Li, D. Li, L. Chen, W. Jiang, Y. Kitamura, B. Li, Volatile organic acid adsorption and cation dissociation by porphyritic andesite for enhancing hydrolysis and acidogenesis of solid food wastes, Bioresource Technology, 101 (2010) 5076-5083.
[98] Y.F. Wang, F. Lin, W.Q. Pang, Ammonium exchange in aqueous solution using Chinese natural clinoptilolite and modified zeolite, Journal of Hazardous Materials, 142 (2007) 160-164.
[99] M. Li, X. Zhu, F. Zhu, G. Ren, G. Cao, L. Song, Application of modified zeolite for ammonium removal from drinking water, Desalination, 271 (2011) 295-300.
[100] C.A. Jackson-Moss, J.R. Duncan, D.R. Cooper, The effect of calcium on anaerobic digestion, Biotechnology Letters, 11 (1989) 219-224.
[101] J.H. Ahn, T.H. Do, S.D. Kim, S. Hwang, The effect of calcium on the anaerobic digestion treating swine wastewater, Biochemical Engineering Journal, 30 (2006) 33-38.
[102] R. Le van Mao, L. Lu, N. Vu, Q. Zhao, H. Yan, N. Al-yassir, Silica nanoboxes from alumina rich zeolites: thermal and chemical stability of the monomodal and bimodal materials, Catalysis Letters, 114 (2007) 129-134.
[103] H.M.F. Freundlich, Uber die adsorption in losungen, zeitschrift fur phtsikalische chemie (leipzig), 57A (1906).
[104] I. Langmuir, The adsorption of gases on plane surfaces of glass mica and platinum, Journal of the American Chemical Society, 40 (1918) 1361-1367.
[105] M. Zhang, H. Zhang, D. Xu, L. Han, D. Niu, L. Zhang, W. Wu, B. Tian, Ammonium removal from aqueous solution by zeolites synthesized from low-calcium and high-calcium fly ashes, Desalination, 277 (2011) 46-53.
[106] L. Lei, X. Li, X. Zhang, Ammonium removal from aqueous solutions using microwave-treated natural Chinese zeolite, Separation and Purification Technology, 58 (2008) 359-366.
[107] H. Huang, X. Xiao, B. Yan, L. Yang, Ammonium removal from aqueous solutions by using natural Chinese (Chende) zeolite as adsorbent, Journal of Hazardous Materials, 175 (2010) 247-252.
[108] Y.S. Ho, G. McKay, Pseudo-second order model for sorption processes, Process Biochemistry, 34 (1999) 451-465.
[109] S.Lagergren, About the theory of so-called adsorption of soluble substances, Kung Sven Veten Hand, 24 (1989) 1-39.
[110] A. Hedstrom, Ion exchange of ammonium in zeolites: a literature review, Journal of Environmental Engineering, 127 (2001) 673-681.
[111] A.D. Eaton, L.S. Clesceri, E.W. Rice, E. Arnold, Standard methods for the examination of water and wastewater., American Public Health Association, 2005.
[112] F. Yan, Study on the Soaking-out of Elements from Weihui Maifanite, Rock and mineral analysis, 18 (1999) 142-145.
[113] M. Takashima, R.E. Speece, Mineral requirements for methane fermentation, Critical Reviews in

Environmental Control, 19 (1990) 465-479.
[114] P. Smigan, P. Rusnak, M. Greksák, T.N. Zhilina, G.A. Zavarzin, Mode of sodium ion action on methanogenesis and ATPase of the moderate halophilic methanogenis bacterium Methanohalophilus halophilus, FEBS Letters, 300 (1992) 193-196.
[115] R. Hensel, H. König, Thermoadaptation of methanogenic bacteria by intracellular ion concentration, FEMS Microbiology Letters, 49 (1988) 75-79.
[116] P. Van der Meijden, B.W. Te Brommelstroet, C.M. Poirot, C. van der Drift, G.D. Vogels, Purification and properties of methanol:5-hydroxybenzimidazolylcobamide methyltransferase from Methanosarcina barkeri, Journal of Bacteriology, 160 (1984) 629-635.
[117] J.E. Schmidt, B.K. Ahring, Effects of magnesium on thermophilic acetate-degrading granules in upflow anaerobic sludge blanket (UASB) reactors, Enzyme and Microbial Technology, 15 (1993) 304-310.
[118] Z. Chen, J. Potempa, A. Polanowski, M. Wikstrom, J. Travis, Purification and characterization of a 50-kDa cysteine proteinase (gingipain) from Porphyromonas gingivalis, The journal of biological chemistry, 267 (1992) 18896-18901.
[119] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: A review, Bioresource Technology, 99 (2008) 4044-4064.
[120] S. Uludag-Demirer, G.N. Demirer, C. Frear, S. Chen, Anaerobic digestion of dairy manure with enhanced ammonia removal, Journal of Environmental Management, 86 (2008) 193-200.
[121] M. Lange, B.K. Ahring, A comprehensive study into the molecular methodology and molecular biology of methanogenic Archaea, FEMS Microbiology Reviews, 25 (2001) 553-571.
[122] W.-C. Kuo, T.-Y. Shu, Biological pre-treatment of wastewater containing sulfate using anaerobic immobilized cells, Journal of Hazardous Materials, 113 (2004) 147-155.
[123] F. Vedrenne, F. Béline, P. Dabert, N. Bernet, The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes, Bioresource Technology, 99 (2008) 146-155.
[124] L. Zhang, D. Jahng, Enhanced anaerobic digestion of piggery wastewater by ammonia stripping: Effects of alkali types, Journal of Hazardous Materials, 182 (2010) 536-543.

Acknowledgements

I would first like to thank my advisor, Professor Zhenya Zhang, for providing me the opportunity to undertake this research, for his enthusiasm, encouragement, determination and depth of knowledge. I also wish to express my appreciation to my co-supervisor, associate professor Yingnan Yang, for her guidance, expertise and friendship during my doctor studies. Her professionalism and hardworking is an excellent example I can follow throughout my career.

I also would like to express my great appreciation to my thesis committee members, Professor Zhenya Zhang, Norio Sugiura, Yingnan Yang and Yoshiro Higano for their patient reading and listening, numerous suggestions and comments to serve as committee members. All their instructors have provided great insight and a memorable learning experience at University of Tsukuba that I will remember for the rest of my life.

I also thank Professor Norio Sugiura and associate professor Motoo Utsumi for providing access to their laboratory for their support throughout my dissertation research.

I would also like to thank Professor Chuanping Feng in China University of Geosciencs for giving me motivation to pursue higher education at University of Tsukuba that most students will never have the chance to encounter.

Many friends and fellow students deserve recognition for their direct and indirect contributions to this thesis. I wish to express my appreciation to Cang Yu, He Huang, Dawei Li, Leilei He, Yingxin Zhao, Yaning Wang, Chunguang Liu, Min shi, Yiting Li, Jinglu Liu, Xu guo, Chao Zhao, Chengjie Han, and many others for their countless assistance during the three years of my doctor study.

Finally, I would like to thank my parents and my friend Peng Zhao. This dissertation would not have been completed without their love and support.

Appendix

1. **Qinghong Wang,** Chuanping Feng, Yingxin Zhao, Chunbo Hao. Denitrification of Nitrate Contaminated Groundwater with a Fiber-based Biofilm Reactor. Bioresource Technology, Volume 100, Issue 7, April 2009, Pages 2223-2227**(IF,4.364)**
2. **Qinghong Wang**, Yingnan Yang, Cang Yu, He Huang, Mijung Kim, Chuanping Feng, Zhenya Zhang. Study on a fixed zeolite bioreactor for anaerobic digestion of ammonium-rich swine wastes. Bioresource technology. Volume 102, Issue 14, July 2011, Pages 7064-7068 (**IF,4.364**)
3. **Qinghong Wang,** Yingnan Yang, Dawei Li, Chuanping Feng, Zhenya Zhang. Treatment of ammonium-rich swine waste in modified porphyritic andesite fixed-bed anaerobic bioreactor, Bioresource Technology, In Press, Available online 8 February 2012. (**IF,4.364**)
4. **Qinghong Wang**, Chuanping Feng, Yingxin Zhao, Chunbo Hao . Nitrate Removal from Groundwater by a Biofilm Reactor. Conference on Advances in Chemical Technologies for Water and Wastewater Treatment (CCS-XIAN2008),xi'an,China Shan xi Science and Technology Press 625-632 **(ISTP**)
5. **Qinghong Wang,** Yingnan Yang, Dawei Li, Chuanping Feng, Zhenya Zhang. Evaluation of a Ca-modified porphyritic andesite for ammonium removal in the anaerobic digestion process, Envionmental Technology, under review. (**IF,1.007**)
6. Yingxin Zhao, Chuanping Feng, **Qinghong Wang**, Yingnan Yang, Zhenya Zhang, Norio Sugiura Nitrate removal from groundwater by cooperating heterotrophic with autotrophic denitrification in a biofilm–electrode reactor, Journal of Hazardous Materials, Volume 192, Issue 3, 15 September 2011, Pages 1033-1039 (**IF:3.723**)
7. Mijung Kim, Yingnan Yang, Marino S. Morikawa-Sakura, **Qinghong Wang**, Michael V. Lee, Dong-Yeol Lee, Chuanping Feng, Yulin Zhou, Zhenya Zhang. Hydrogen production by anaerobic co-digestion of rice straw and sewage sludge. International journal of hydrogen energy. Volume 37, Issue 4, February 2012, Pages 3142-3149 (**IF,4.052**)

8. Min Shi, Yingnan Yang, **Qinghong Wang**, Ying Zhang, Yuepeng Wang, Zhenya Zhang.Optimum Condition of Fermentation by Lentinus edodes Using Soybean C urd Residue as Raw Materials. Journal of food science and technology (**IF:0.477**)
9. Tomonao Miyashiro, **Qinghong Wang**, Yingnan Yang, Kazuya Shimizu, Noria Sugiura, Sabro Matsui. High efficient anaerobic digestion of refractory organic waste using subcritical water hydrolysis as pretreatment, J-STAGE, Japan Science and Technology Information Aggregator, Electronic Vol.48, No.1, March 2012